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SOME OBSERVATIONS REGARDING EYE-LENS WEIGHT AS A CRITERION OF AGE IN ANIMALS[1]

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ABSTRACT

Studies of specimens from white rats demonstrated that eye-lens weight increases as an animal grows older. Curves for both wet and dry weight, and for fresh as well as formalin-fixed specimens, gave equally usable indices. Measurements of specific gravity also reflected age differences. Fixing the eye in formalin, followed by lens removal, ovendrying and weighing, was found to be the best method. The length of time in formalin must be considered when evaluating data obtained in this way. Tests with white rats and guinea pigs indicated that, under field conditions, quick freezing may be used to preserve lenses until they can be placed in formalin or processed further. The procedure for removing the eye and processing the lens is described.

Lord (1959) described a technique for determining the age of cottontail rabbits by using the dry weight of the eye lens. Since his initial work, considerable interest has been shown in using the technique for other wildlife species (Friend, 1967), but little modification or study of associated variables has been undertaken. Fixation in formalin prior to ovendrying and weighing is the accepted method of processing, yet investigations concerning the effects of formalin on lens weights have not been repotred. Montgomery (1963) and Longhurst (1964) both studied the effects of freezing on lens weights.

To learn more about variations in eye-lens weights, as well as variables associated with the techniques, and to provide information on the interpretation of lens data, the author undertook studies in 1965 and 1966. They were carried on largely at the Griffin Laboratory of the New York State Health Department, although some work was done at the Harvard Medical School and at the Wildlife Research Laboratory at Delmar (N. Y.). Albino rats and guinea pigs were used in the experiments conducted. The findings are

[1] A contribution of Federal Aid in Fish and Wildlife Restoration Project W-35-R. The photographs were made by Nicholas Drahos of the New York State Conservation Department.

reported here. However, a brief description of the eye and procedures for handling specimens is given first.

ANATOMY OF THE EYE

A logical starting point in developing a technique for lens removal is to review the structure of the eye. In the following discussion the eye is dissected one layer at a time starting with the outside covering and ending with the internal refractory bodies. General location is noted for all major parts and more detailed reference is made to those structures directly involved in the lens technique.

The eye may be thought of in terms of three major components: the eyeball, the optic nerve, and associated accessory organs. Only the eyeball will be considered here; for a more complete and detailed

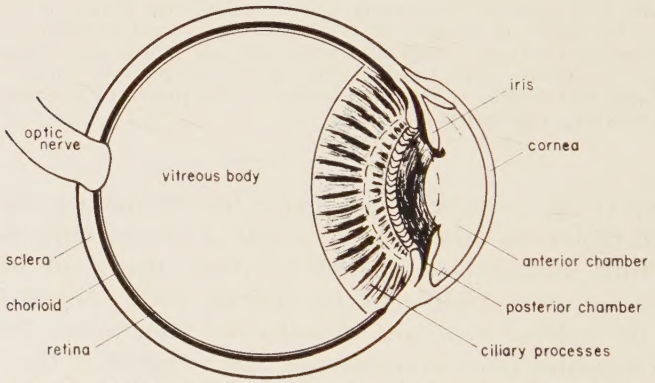

Figure 1. Simplified diagram of a longitudinal section through the eye.

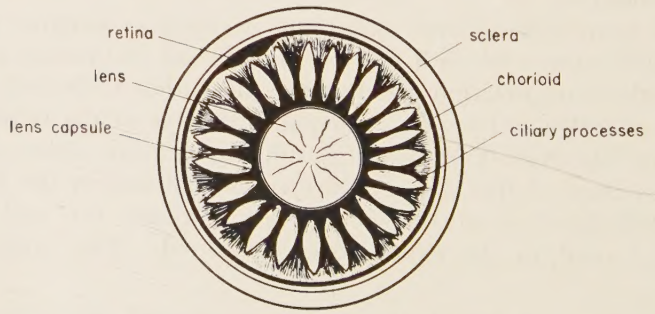

Figure 2. Simplified diagram of a cross-section through the eye posterior to the lens.

discussion consult the work of Sisson and Grossman (1962, pp. 879-892) or Walls (1963).

Examination of the eyeball reveals three concentric coats or layers: the tough outer fibrous tunic; the middle vascular tunic; and the internal neuroepithelial tunic. Each of these layers is composed of one or more major structures, and in combination they enclose the anterior and posterior chambers, lens, and vitreous body (Figure 1).

Fibrous Tunic

This thick, tough outside layer gives the eye its form and protects the rather delicate inner structures. It is composed of two sections: the sclera, which is dense and fibrous and forms the posterior four-fifths; and the cornea, which is transparent, colorless and non-vascular and forms the anterior fifth. The chambers are filled by aqueous humor, a clear fluid consisting of about 98 per cent water. In contrast, the vitreous body, a semifluid transparent substance filling the space between the lens and the retina, consists of a framework of delicate fibrils known as the vitreous stroma. The meshes of this stroma are filled by the clear fluid—the vitreous humor.

Vascular Tunic

Internal to the fibrous tunic is the layer responsible for the nutrition of the ocular tissues. Also, the smooth muscles of this layer provide the mechanism for accommodation. The chorioid, ciliary body and iris are found here.

The chorioid is the thin membrane lying between the sclera and the retina. It is of no concern in the lens technique.

The ciliary body is the complex structure connecting the chorioid with the periphery of the iris and is composed of the ciliary ring, ciliary processes and ciliary muscle. The ciliary processes are prominent as radial folds forming a circle around the lens (Figures 1 and 2). Portions may adhere to the lens when it is being removed.

The iris is the muscular diaphragm in front of the lens and visible through the cornea. It is pierced centrally by an elliptical opening—the pupil.

Neuroepithelial Tunic

This is the innermost layer and takes the form of a delicate membrane known as the retina. It extends from the entrance of the optic nerve to the margin of the pupil. Contained within this structure are the receptors for light and the first links of nervous pathways that convey impulses through the optic nerve to the brain.

Lens

The crystalline lens is a transparent, round, biconvex body lying in front of the vitreous body and in partial contact with the posterior surface of the iris. It continues to grow throughout the entire adult life of an animal as a result of division of a single layer of cells over its anterior surface. The solid matter of the lens is about 35 per cent of the weight of the whole, and nearly all of this is protein.

Covering the lens proper is a thin, highly elastic, homogenous, transparent membrane—the lens capsule. The thickness of this capsule varies with the animal and location of measurement on the capsule. Generally, the bigger the lens, the thicker the capsule. In animals, thicknesses of 1 to 80 microns have been reported. It is interesting to note that the capsule increases in thickness with the age of the lens.

GENERAL PROCEDURES FOR HANDLING SPECIMENS

Since this is a relatively new technique, a description of the equipment and procedures used for handling specimens is included. Use of specific gravity has not been reported with respect to wildlife species.

Equipment

Processing can be made easier by selecting proper equipment. The following were used by the author, but do not include the specialized equipment needed for determining specific gravity.

Magnifying lamp with 3× eye piece
Straight and curved iris forceps
Straight and curved iris scissors
Riggs stadie blade
Squares of heavy blotting paper—4 x 4 inches
Strips of heavy blotting paper—3 x ¾ inches
Shell vials
Drying rack
Thelco mechanical convection oven
Mettler single-pan analytical balance (Model B)

Use of a magnifying lamp facilitates removing the lens from animals with small eyes. Eye strain and fatigue are materially reduced, and the worker can satisfactorily remove a greater number of lenses during a given time period.

Curved scissors were found best for removing the eye and straight scissors best for opening the sclera.

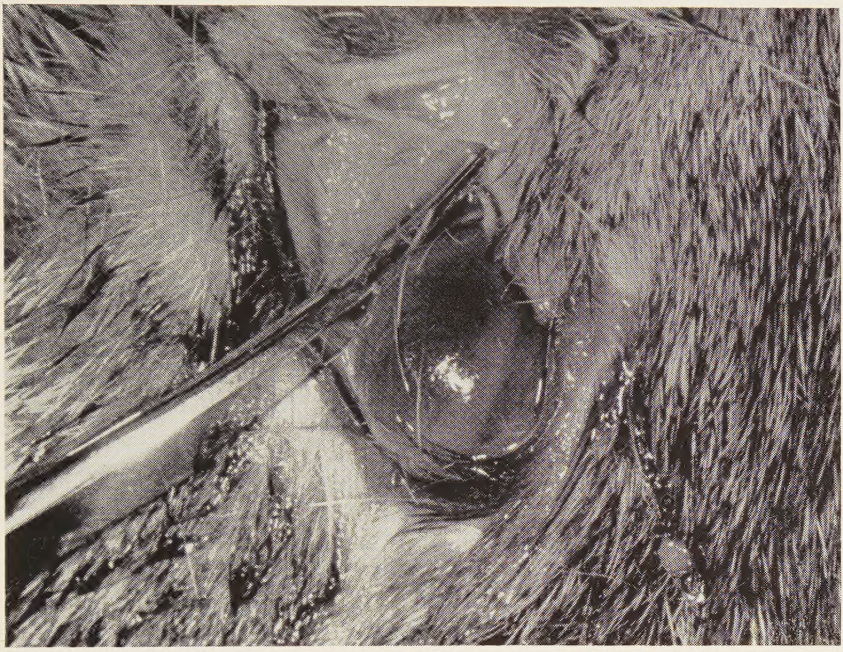

Figure 3. Cutting the eyelids to facilitate removal of the eye.

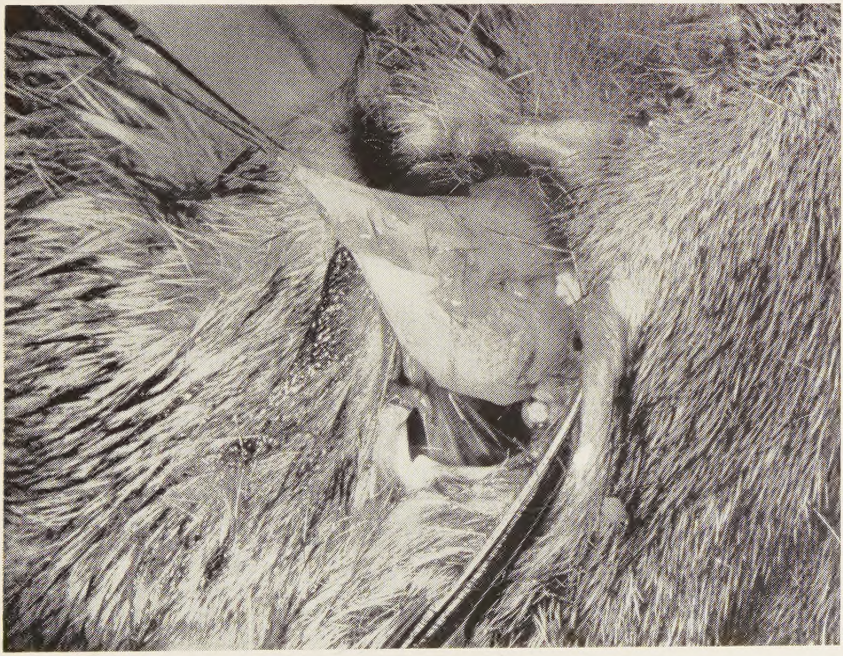

Figure 4. Freeing the eye from its attachment.

The stadie blade is used only for making the initial cut through the sclera. This thin steel blade is superior to either a scalpel or razor blade because it is easier to handle and has a sharp cutting edge of almost 5 inches.

Blotting or filter paper should be relatively stiff. Cut in strips, it is used to tease the lens out of the opened eye; squares of it are used to roll the lens on for removing any adhering ciliary body and to blot aqueous and vitreous fluids from the lens.

The circular drying rack used was designed to fit in a desiccator. Wooden drying racks should be ovendried for several days before initial use to remove resins from the wood which might collect on the vials during the drying process.

A single-pan analytical balance greatly increases the speed of the weighing operations with no loss in accuracy. It is reliable, accurate and simple to operate.

Removing Eye and Lens

The first step is to remove the eyeball from the animal intact in the following manner.

1. Enlarge the orbit by cutting at the corners of the eyelids as shown in Figure 3.
2. Roll the eye outward in its socket and, holding it with forceps, cut it free of its attachments (Figure 4).

If it is to be fixed, place the entire eye in 10 per cent formalin. For large eyes, such as those of deer, making a small slit in the vicinity of the optic nerve will allow better penetration of the fixative.

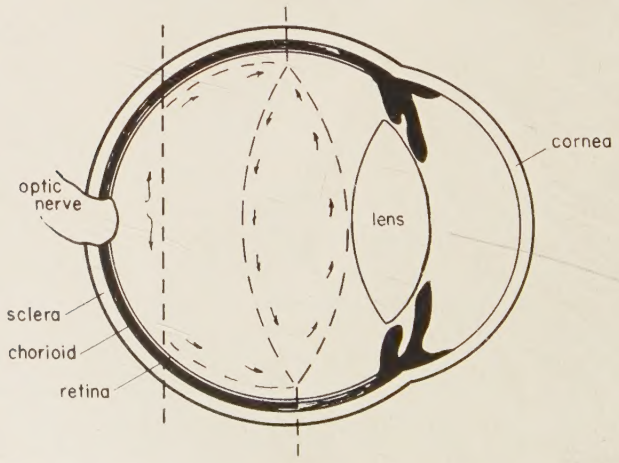

Figure 5. Diagram of cuts used in dissecting the lens from the eye.

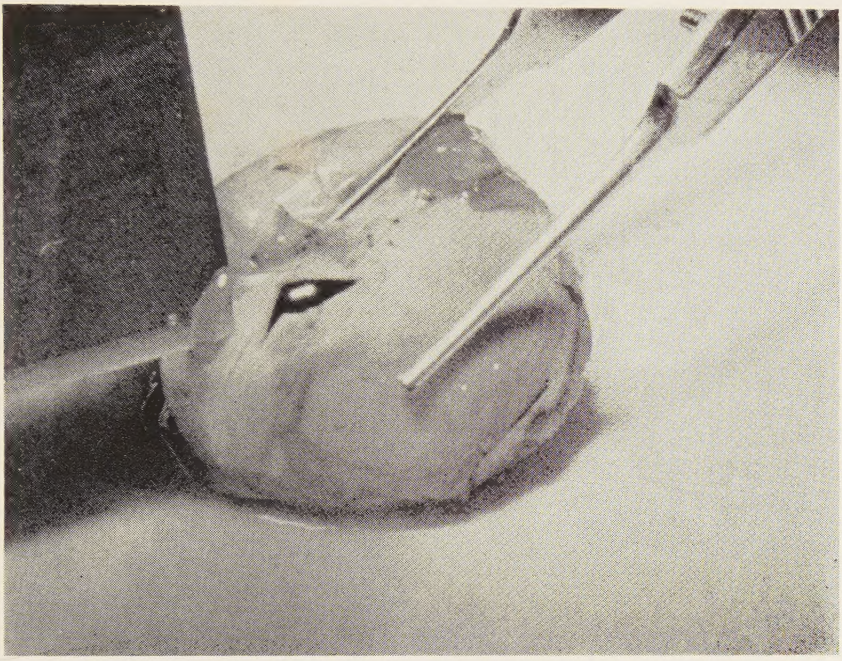

Figure 6. Initial incision through the sclera, made for removing the lens from the eye.

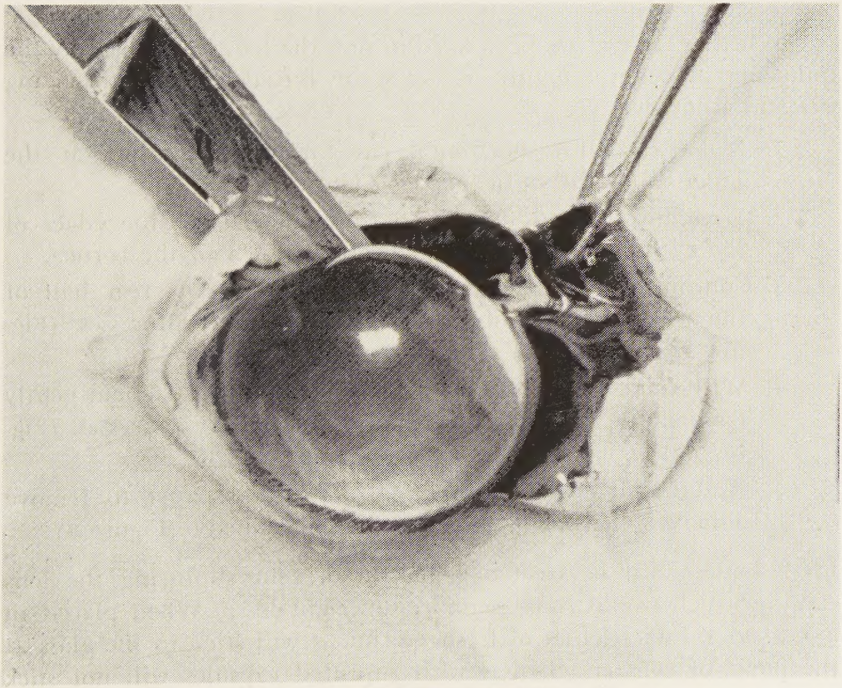

Figure 7. Freeing the lens from its ciliary processes.

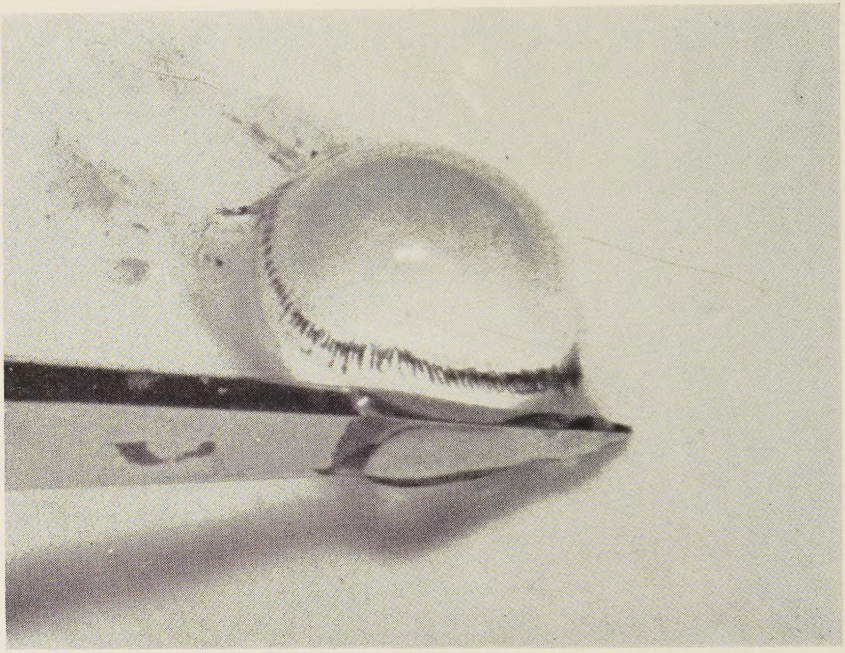

Figure 8. A lens with portions of the ciliary ring attached.

Whether the eye has been fixed or not, the lens is removed by the following procedure; Figure 5 shows the initial steps in dissecting the lens from the eye.

1. Make a shallow horizontal cut through the sclera at the junction of the optic nerve (Figure 6).
2. With forceps and scissors continue the cut to the edges of the eyeball, being careful not to cut down to the cornea.
3. Continue the cut around the eye removing the rear half of the sclera and exposing the lens and ciliary processes (Figure 2).
4. With folded strips of filter paper or a blunt instrument gently tease the lens free from the adjoining ciliary processes (Figure 7).
5. Roll the lens gently on filter or blotting paper to remove adhering vitreous or traces of the ciliary body (Figure 8).

Fresh lenses must be treated gently to prevent rupturing the lens capsule which would cause significant weight loss. When placed in a glass vial, intact lenses of fresh specimens will stick to the glass at the point of contact. Lenses with ruptured capsules will not stick

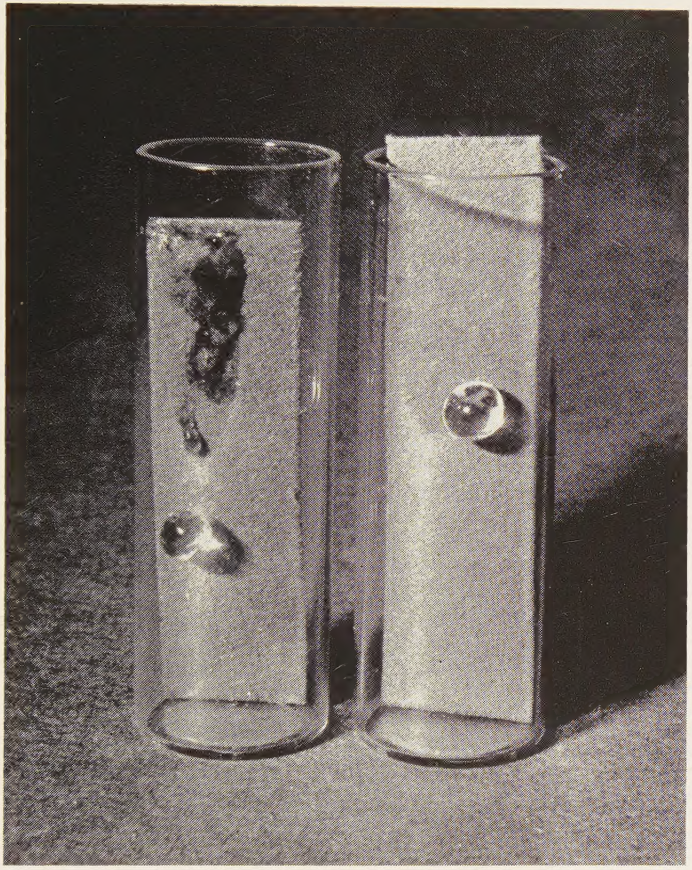

Figure 9. Comparison between a ruptured (left) and non-ruptured (right) eye lens.

but slowly slide down the side of the vial (Figure 9). This test cannot be used with fixed lenses.

WEIGHING

Lenses may be weighed fresh or after being fixed in formalin. In either case, they may be weighed wet or after being ovendried. Weights are usually taken to the nearest 0.1 milligram on an analytical balance.

DRYING

An electric oven is used to obtain dry weights. Lenses should be dried in open vials or in loosely covered or open drying dishes. A lens is considered dry when further significant weight loss does

TABLE 1. CHANGES IN WEIGHT FOR OVENDRIED FRESH LENSES AND SHELL VIALS AFTER EXPOSURE AT ROOM TEMPERATURE FOR DIFFERENT INTERVALS

Treatment	Weight in milligrams					
	Start	1 minute	2 minutes	5 minutes	7 minutes	10 minutes
Lens only						
Transferred from oven to cool vial on balance	4,057.8	4,057.8	4,057.8	4,057.8	4,057.8	4,057.8
Variation*	..	0.0	0.0	0.0	0.0	0.0
Transferred from oven to cool vial on balance; lens and vial removed between weighings	4,058.5	4,058.5	4,058.6	4,058.7	4,058.7	4,058.7
Variation*	..	0.0	+0.1	+0.2	+0.2	+0.2
Lens and vial						
Transferred from oven to balance	4,107.1	4,107.8	4,108.3	4,109.0	4,109.3	4,109.4
Variation*	..	+0.7	+1.2	+1.9	+2.2	+2.3
Vial only						
Transferred from oven to balance	4,075.3	4,075.7	4,076.3	4,076.8	4,077.0	1,077.1
Variation*	..	+0.4	+1.0	+1.5	+1.7	+1.8
Transferred from oven to balance	4,021.2	4,021.8	4,022.2	4,022.8	4,023.0	1,023.1
Variation*	..	+0.6	+1.0	+1.6	+1.8	+1.9
Removed from oven, cooled 2 minutes, then weighed	3,999.6	..	3,998.6	..	..	..
Variation*	..	..	−1.0	..	..	..
Removed from oven, cooled 5 minutes, then weighed	4,156.1	..	..	4,155.8	..	..
Variation	..	..	..	−0.3	..	..
Removed from oven, cooled 10 minutes, then weighed	4,020.5	..	..	..	..	4,020.4
Variation	..	..	..	..	..	−0.1

* Cumulative.

not occur with additional drying time. For animals with small lenses 24 hours at 120° C. will usually be sufficient, but lenses from larger animals, such as deer, may require from several days to a week. The low temperatures recorded by some authors are not necessary and require longer oven time. Some splitting may occur with fixed lenses if too high a temperture is used, but the writer has had good success with albino rat lenses dried at 120° C. for 16 to 18 hours.

It was found that the dried lens is not hygroscopic as others have believed. However, the glass vials in which lenses were dried were

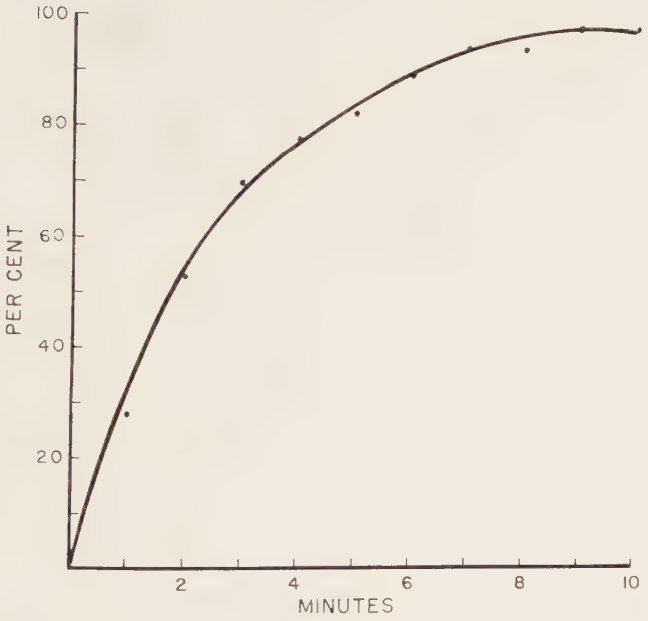

Figure 10. Rate (in per cent) at which vials regained weight after removal from the drying oven.

found to lose weight during the drying process and then to regain the loss as they cooled (Table 1 and Figure 10). Hence, vials should be thoroughly cooled before lens weighings are made; data indicated that for all practical purposes weight gain ceased after 10 minutes.

DETERMINING SPECIFIC GRAVITY[2]

Weight relationships may also be derived from values for specific gravity. Lowry and Hunter (1945) used such a technique for obtaining rapid and precise measurements of serum protein concentration, and it was adopted as a measure of lens weight by Hart and Peckham (1953).

The technique consists of using mixtures having different proportions of a heavy and a light organic liquid (bromobenzine and kerosene) to establish a linear density gradient in a graduated cylinder. Droplets of salt solutions of known density (standards) are allowed to fall into this gradient solution, and each comes to rest when it reaches the point having a density exactly equal to its own. These points are then read with an Eberbach cathetometer by

[2] The writer was instructed and assisted in this technique by personnel of the Harvard Medical School engaged in eye research.

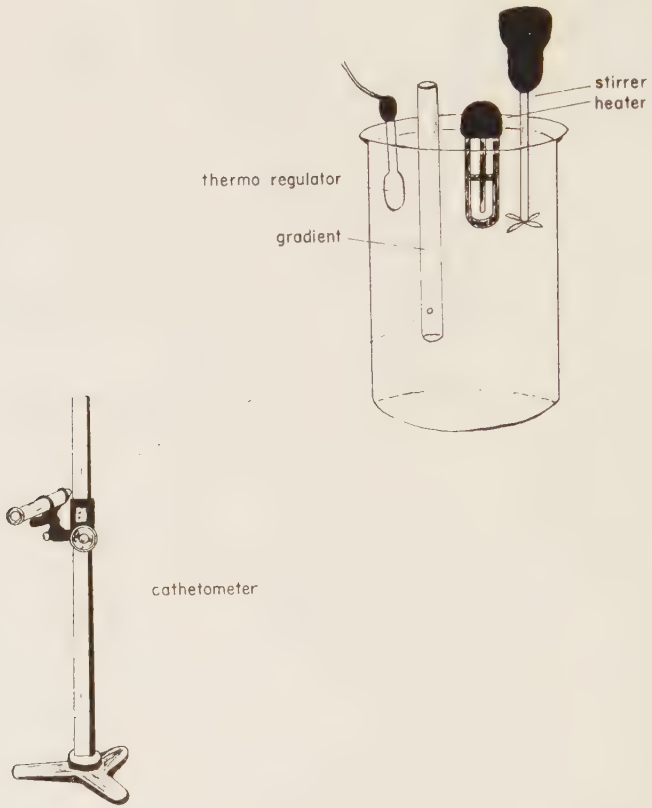

Figure 11. Diagram of equipment used for determining the specific gravity of a lens.

placing the center of the cross hairs at the center of the drop being measured. The readings obtained are plotted to check the linearity of the column and also to serve as the base line for interpolating the specific gravities of lenses being studied. Hart and Peckham gave the accuracy of the method as ± 0.0002. A simplified drawing of the apparatus used is shown in Figure 11. For a more detailed presentation of the method and apparatus see Lowry and Hunter (1945). However, this method is applicable to fresh specimens only.

In the work reported here, a gradient solution with a total volume of approximately 200 milliliters was prepared as described in Table 2. Its linearity was determined by dropping into it from the end of a capillary pipet a single bubble from each of six concentrations of potassium chloride, i.e. 24, 22, 20, 18, 16 and 14 per cent. The point at which each bubble stopped was read in centi-

TABLE 2. AMOUNTS OF BROMOBENZINE AND KEROSENE NEEDED TO FORM A GRADIENT SOLUTION WITH A TOTAL VOLUME OF APPROXIMATELY 200 MILLILITERS

Dilution	Ratio of mixture*	Volume in milliliters§
1	5.0/4.3	8.75/7.50
2	4.9/4.4	8.60/7.70
3	4.8/4.5	8.40/7.86
4	4.7/4.6	8.23/8.05
5	4.6/4.7	8.05/8.23
6	4.5/4.8	7.86/8.40
7	4.4/4.9	7.70/8.60
8	4.3/5.0	7.50/8.75
9	4.2/5.1	7.35/8.95
10	4.1/5.2	7.17/9.10
11	4.0/5.3	7.00/9.25
12	3.9/5.4	6.84/9.45

* Bromobenzine/kerosene.
§ Ratio × 1.75, factor to give approximately 200 milliliters.

meters and plotted against the known specific gravity of that concentration of potassium chloride at 30° C. (temperature maintained). This procedure was followed each day before any lenses were processed.

To obtain the specific gravity of a lens specimen, it was placed gently into the gradient solution, and the point to which it settled was read and plotted against the established base line. The lens was not removed from the cylinder after the reading was taken but was settled to the bottom by sprinkling fine sand (60-80 mesh) on it. The sand particles adhered to the lens and carried it to the bottom leaving the gradient solution ready for use again immediately. A new gradient solution was prepared when the one in use became unstable or the collection of lenses and sand at the bottom became too high.

COMPARISON OF DATA FOR LENSES PROCESSED BY DIFFERENT METHODS

Representative series of eye lenses from known-age albino rats of various ages were removed from the animals immediately after death and either processed fresh or fixed in 10 per cent formalin prior to being weighed on a balance. In each case, part of the specimens were weighed wet and part after they had been ovendried. The data are given in Tables 3 to 6, and graphic comparisons are made in Figures 12 and 13.

It is evident from these data that lens weight increased with age. The differential was greater for wet specimens than for dried speci-

Table 3. Wet Weights of Fresh Eye Lenses from Known-age Albino Rats

Age in days	Weight in milligrams			
	Male		Female	
	Mean*	Range	Mean*	Range
24	..	..	16.2 (3)	16.1–16.3
28	17.5 (9)	16.5–18.2	17.0 (8)	16.1–17.9
32	20.6 (10)	16.7–21.7	20.5 (9)	16.1–22.9
36	22.0 (9)	20.1–23.6	..	..
37	22.3 (7)	20.7–23.4	20.6 (8)	18.4–23.1
38	23.3 (5)	22.2–24.4	21.9 (5)	21.5–22.3
41	24.5 (5)	23.5–26.2	..	..
43	..	..	26.0 (7)	25.1–27.2
47	..	..	24.6 (8)	20.8–27.2
57	29.1 (12)	28.0–29.9	..	..
60	32.1 (10)	27.9–36.1	32.6 (10)	30.3–34.5
62	31.8 (9)	30.3–34.3	30.6 (11)	28.4–33.5
64	30.9 (10)	29.4–32.8	..	..
65	31.8 (8)	30.2–32.8	..	..
66	32.5 (10)	30.8–34.0	30.5 (9)	27.5–31.9
67	..	..	30.4 (7)	29.7–30.9
68	..	..	31.7 (5)	30.7–32.8
70	..	..	31.6 (4)	29.9–32.5
72	32.4 (5)	32.0–33.5	32.5 (5)	30.7–33.6
79	36.3 (5)	35.2–37.4	..	..
82	..	..	37.2 (5)	33.6–43.8
93	37.9 (11)	36.3–40.8	..	..
109	..	..	40.3 (11)	38.0–60.1
119	..	..	42.0 (6)	40.0–45.4
120	43.8 (12)	39.9–48.0	42.2 (6)	41.0–43.6
150	..	..	46.9 (9)	43.6–48.8
153	46.6 (4)	45.1–47.3	..	..
157	..	..	47.8 (6)	46.0–49.6
159	..	..	46.4 (8)	43.0–48.8
171	..	..	47.6 (16)	44.6–50.9
178	..	..	47.9 (5)	46.5–48.9
181	53.6 (4)	50.5–56.6	..	..
195	..	..	45.8 (3)	44.4–46.8
227	54.5 (4)	53.4–56.2	..	..

* Figures in parentheses represent number of specimens.

mens, but in each case curves for fixed and fresh lenses closely paralleled each other. Lenses of males were heavier than those of females. When the individual values were plotted, the weights of dried lenses appeared to cluster more closely than those of wet lenses. However, the degree of variation from the mean did not differ significantly for most age groups.

To compare the four methods of treatment, three age groups represented in the data for all four were selected at random (Table 7). For wet specimens, the mean weights of fixed lenses were less than those of fresh lenses, but the reverse was true for dried specimens. The greatest variations from the mean occurred in the youngest

TABLE 4. DRY WEIGHTS OF FRESH EYE LENSES FROM KNOWN-AGE ALBINO RATS

Age in days	Weight in milligrams			
	Male		Female	
	Mean*	Range	Mean*	Range
24	..	..	5.8 (3)	5.7– 6.0
28	6.8 (9)	6.5– 7.4	6.6 (8)	6.3– 6.9
32	8.0 (10)	7.3– 8.4	7.9 (9)	6.7– 8.3
36	8.5 (9)	7.7– 9.1	..	..
37	8.4 (7)	7.6– 8.8	7.9 (8)	7.0– 9.4
38	8.7 (5)	7.6– 9.4	8.4 (5)	8.2– 8.7
41	9.8 (5)	9.2–10.8	..	..
43	..	..	9.8 (7)	9.2–10.1
47	..	..	10.2 (8)	9.4–11.2
57	11.2 (12)	10.9–12.0	..	..
60	13.3 (10)	12.7–14.2	13.3 (10)	12.4–13.9
62	12.7 (9)	12.2–13.3	12.5 (11)	11.1–14.1
64	12.9 (10)	12.1–13.6	..	..
65	12.9 (8)	12.2–13.6	..	.....
66	13.0 (10)	12.4–13.8	12.8 (9)	12.1–14.0
67	..	..	12.2 (7)	11.8–12.6
68	..	..	12.5 (5)	12.1–12.9
70	..	..	12.8 (4)	12.2–13.6
72	13.1 (5)	12.6–13.5	13.3 (5)	12.6–14.2
79	14.9 (5)	14.3–15.9	..	..
82	..	..	15.0 (5)	14.0–16.2
93	15.7 (11)	15.1–16.7	..	..
109	..	..	16.9 (11)	16.0–27.6
119	..	..	17.6 (6)	17.2–18.5
120	18.1 (12)	17.7–18.5	18.0 (5)	17.5–18.6
150	..	..	19.8 (9)	19.0–20.6
153	20.7 (4)	20.0–21.1	..	..
157	..	..	20.5 (6)	19.7–21.5
159	..	..	20.3 (8)	18.8–21.4
171	..	..	20.9 (16)	19.6–22.2
178	..	..	21.1 (5)	20.7–22.0
181	23.8 (4)	23.1–24.3	..	..
195	..	..	20.6 (3)	20.5–20.7
227	24.4 (4)	23.5–25.4	..	..

* Figures in parentheses represent number of specimens.

group, and the least in the oldest age group (Table 8). There was no difference between fresh and fixed lenses in the degree of variation. For males, weights of fixed lenses generally exhibited a greater variation than those of fresh lenses, while for females weights of fresh lenses varied more.

With respect to specific gravity, measurements for a series of lenses were made at the Howe Laboratory of Ophthalmology of the Harvard University Medical School. The data showed considerable variation within any given age group (Figure 14). Along with this variation wide overlaps between animals of dissimilar age groups occurred. While measurement by this method is very precise

Table 5. Wet Weights of Formalin-fixed Eyes Lenses from Known-age Albino Rats

Age in days	Weight in milligrams			
	Male		Female	
	Mean*	Range	Mean*	Range
28	15.9 (10)	15.0–17.2	15.9 (10)	15.4–16.8
36	21.4 (9)	19.7–23.1	..	..
37	21.5 (10)	19.3–22.6	19.8 (8)	17.7–21.1
41	22.3 (5)	20.7–23.1	..	..
43	..	..	23.8 (8)	20.7–25.6
47	..	..	24.0 (8)	23.4–25.0
57	26.4 (12)	25.7–27.3	..	..
60	30.8 (11)	28.6–32.4	28.5 (8)	23.8–31.9
62	29.4 (7)	26.9–30.0	28.1 (12)	27.5–31.0
64	29.4 (5)	26.3–30.9	26.2 (6)	25.2–27.5
66	30.2 (15)	28.0–32.0	28.4 (8)	27.1–30.5
67	..	..	27.3 (7)	25.1–28.9
72	28.3 (5)	24.8–30.8	29.5 (5)	28.8–30.7
93	35.5 (14)	34.1–36.6	..	..
109	..	..	36.9 (11)	34.6–54.9
120	40.1 (13)	38.2–41.7	38.7 (6)	26.6–29.9
150	..	..	42.6 (9)	41.5–44.0
153	45.2 (4)	44.3–46.3	..	..
157	..	..	43.0 (6)	40.5–48.4
159	..	..	43.4 (8)	41.1–45.5
178	..	..	45.2 (5)	43.5–48.3

* Figures in parentheses represent number of specimens.

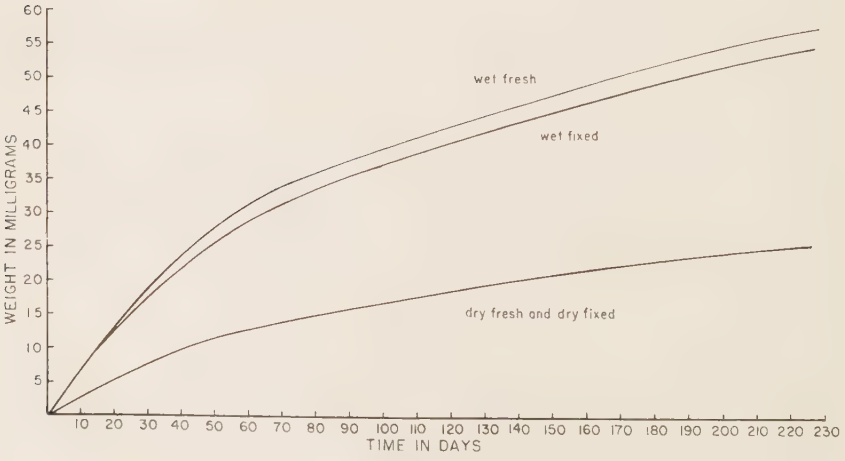

Figure 12. Comparison of wet and dry weights for fresh and fixed eye lenses from male albino rats of different ages.

TABLE 6. DRY WEIGHTS OF FORMALIN-FIXED EYE LENSES FROM KNOWN-AGE ALBINO RATS

Age in days	Weight in milligrams			
	Male		Female	
	Mean*	Range	Mean*	Range
28	6.1 (10)	5.8– 7.1	6.0 (10)	5.7– 6.5
36	8.4 (5)	7.4– 9.5	..	..
37	8.5 (10)	7.5– 9.6	7.6 (8)	6.6– 8.6
41	9.8 (5)	9.1–10.5	..	..
43	..	..	9.7 (8)	8.9–10.2
47	..	..	10.1 (8)	9.3–10.8
57	11.3 (12)	11.0–12.1	..	..
60	13.8 (11)	12.9–14.7	13.4 (8)	12.7–14.0
62	12.8 (7)	12.1–13.2	12.9 (12)	12.2–13.7
64	12.9 (5)	11.2–13.5	12.3 (6)	12.0–12.8
66	13.6 (15)	12.4–14.9	12.8 (8)	12.3–13.5
67	..	..	12.7 (7)	11.2–13.8
72	13.6 (5)	12.8–14.0	13.9 (5)	13.4–14.5
93	16.1 (14)	15.4–17.2	..	..
109	..	..	17.1 (11)	16.4–28.7
120	18.6 (13)	17.8–19.2	18.2 (6)	17.2–18.9
150	..	..	20.4 (9)	19.7–21.4
153	21.2 (4)	20.2–21.9	..	..
157	..	..	20.5 (6)	19.8–22.1
159	..	..	21.0 (8)	20.1–21.9
178	..	..	20.5 (5)	16.8–22.6

* Figures in parentheses represent number of specimens.

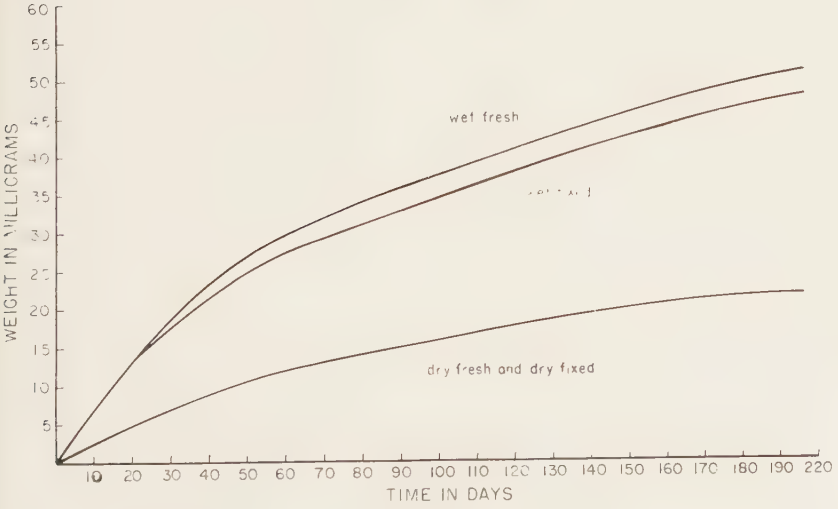

Figure 13. Comparison of wet and dry weights for fresh and fixed eye lenses from female albino rats of different ages.

Table 7. Comparison of Wet and Dry Weights for Fresh and Fixed Eye Lens from Albino Rats

Age in days	Weight in milligrams							
	Wet				Dry			
	Male		Female		Male		Female	
	Mean*	Range	Mean*	Range	Mean*	Range	Mean*	Range
	Fresh							
37	22.3 (7)	20.7–23.4	20.6 (8)	18.4–23.1	8.4 (7)	7.6– 8.8	7.9 (8)	7.0– 9.4
66	32.5 (10)	30.8–34.0	30.5 (9)	27.5–31.9	13.0 (10)	12.4–13.8	12.8 (9)	12.1–14.0
119 & 120	43.8 (12)	39.9–48.0	42.1 (11)	40.0–45.4	18.1 (12)	17.7–18.5	17.8 (11)	17.2–18.6
	Fixed							
37	21.5 (10)	19.3–22.6	19.8 (8)	17.7–21.1	8.5 (10)	7.5– 9.6	7.6 (8)	6.6– .6
66	30.2 (15)	28.0–32.0	28.4 (8)	27.1–30.5	13.6 (15)	12.4–14.9	12.8 (8)	12.3–13.5
120	40.1 (13)	38.2–41.7	38.7 (6)	36.6–39.9	18.6 (13)	17.8–19.2	18.2 (6)	17.2–18.9

* Figures in parentheses represent number of specimens.

TABLE 8. VARIATION FROM THE MEAN FOR WET AND DRY WEIGHTS FOR FRESH AND FIXED EYE LENSES FROM ALBINO RATS

Age in days	Variation in percentage of mean			
	Wet		Dry	
	Male	Female	Male	Female
	Fresh			
37	12.1	22.8	14.3	30.4
66	9.8	14.4	10.8	14.8
120	18.5	12.8	4.4	7.9
	Fixed			
37	15.3	17.2	24.7	26.3
66	13.2	12.0	18.4	9.4
120	8.7	8.5	7.5	9.3

(±0.0002), actual age separations appear to be limited to wider groupings than for either dried or wet lens weights.

In laboratory rats, Hart and Peckham (1953) found no further increase in specific gravity with age after 150 days after fertilization. However, Scammon and Hesdorffer (1937) reported increases in calculated specific gravity for human lenses through the age periods of 20-90 years. Their data were grouped by decade.

Curves for both wet weights and dry weights seem equally satisfactory. Use of wet weights requires more precision and speed in technique than that required when using dry weights. One advantage, however, is that the need for a drying oven is eliminated. Under field conditions where work may be done from a mobile unit, such as a trailer, this makes processing possible.

Similarly, curves for both fresh and fixed lenses seem satisfactory. Depending on circumstances, it may be more expedient to process lenses fresh in the field than to fix them for processing at a later date. Because of the relatively fragile lens capsule, fresh lenses are considerably more difficult to work with than those which have been fixed in formalin. But, as the technician gains experience in working with fresh lenses, few will be ruptured and lost from a sample.

Drying lenses without prior fixation eliminates one important variable, the effects of formalin. Since these lenses must first be handled when wet and fragile, the only advantage to ovendrying them is to reduce variation caused by air drying from exposure before and during the weighing process. Ovendrying is advisable when using a slower-functioning balance than the rapid, direct-reading types.

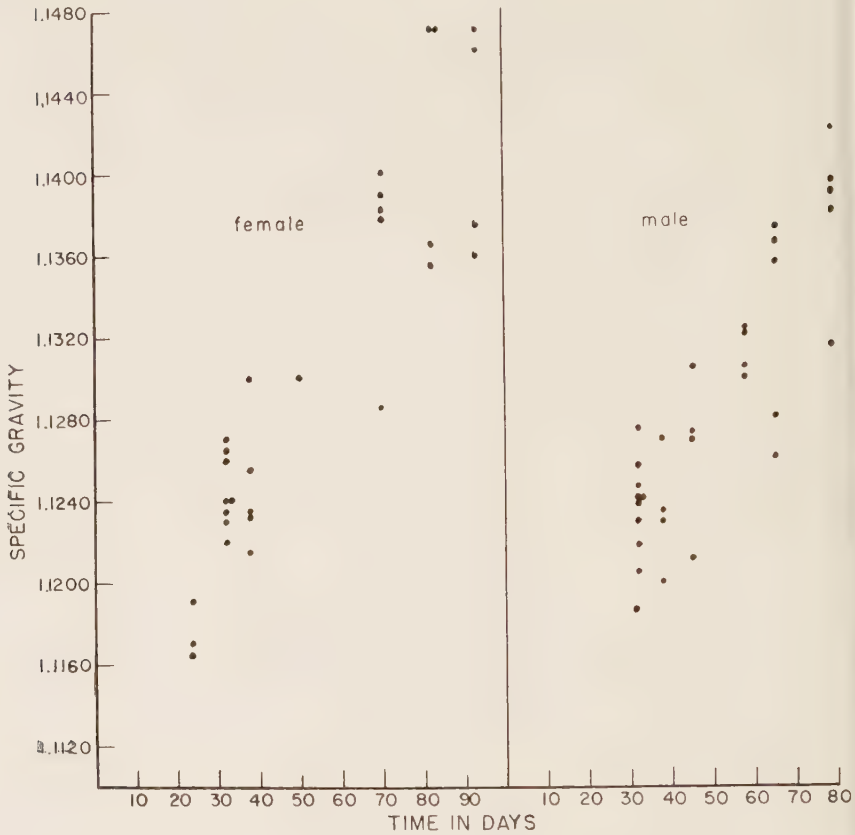

Figure 14. Specific gravity of eye lenses from male and female albino rats.

Fixing lenses in formalin affords the easiest method of handling in many instances, both at the time of collection and later when the lens is removed from the eye. The method is well adapted to field conditions as evidenced by the many studies already conducted on a variety of wildlife species. But it is not without complications. The effect of various concentrations of formalin on lens weights is not known. Little is known regarding the effect of variations in fixation time. It appears likely that some of the variation noted for fixed lenses in this study (Table 8) was the result of unequal fixation times.

Measurement of specific gravity, while used in the field of medicine, has not been used for wildlife species. The technique gives precise measurements but is relatively complex. One obvious advantage is that neither a balance nor oven is required. Other than the cathetometer, the remaining apparatus can be assembled from inexpensive equipment. The adaptability of this technique to wild-

life species needs to be investigated further. Modification of it could provide a relatively rapid field method for determining age in wildlife populations.

For any of the methods described, the conditions under which samples are obtained must be considered. Lenses collected some time after death of the animal may have increased in fresh wet weight due to post mortem absorption of water. In cold climates, freezing the eye and lens may prove to be the best method of field preservation.

Effect of Fixation Time on Lens Weight

Lord (1959) in his original work with lens weights stated that 1 week in 10 per cent formalin was sufficient to fix lenses, but that the longer they remained in the fixative the easier they were to work with. No consideration was given to what effect length of time in the fixative might have on lens weight, and other workers have not provided such information. To test the hypothesis that fixation time need not be considered, a study using laboratory rats was carried out during two consecutive years and the combined findings are presented. During the first year arbitrary fixation periods of 1, 2, 4, 6, 8, 12, 24 and 36 weeks were decided upon for testing, while approximately monthly intervals (30 to 393 days) were used after that.

Albino rats of the Wistar strain were used in groups of 10 or more as they became available from the breeding colony (Table 9). An attempt was made to obtain animals of uniform sex and age, but the number required over the time spans involved made this impossible. The specimens were killed by ether asphyxiation, and both eyes were removed. The lens from one eye was processed immediately (control lens). The remaining eye (treatment lens) was placed in a vial of 10 per cent formalin where it remained until the end of the fixation period to which it was assigned. Selection of lenses for control or treatment was random.

Although all the animals received were used, mechanical injuries to the control lens during processing reduced the number of matched pairs available in many groups. In nearly all instances, specimens were discarded as unusable because the lens capsule was ruptured during removal of an unfixed lens. The high rate of discard among some groups illustrates the difficulty of removing a lens without prior fixation, especially with respect to small animals such as 70-day-old rats.

During the first year, both eyes from specimens assigned to fixation periods of 12, 24 and 36 weeks were intentionally placed in formalin in separate vials. The same applied to the 2-month specimens in the second year, but this was unintentional. These data

Table 9. Distribution of Matched Pairs of Eye Lenses from Albino Rats in Relation to Period of Fixation in Formalin

Period of fixation*	Animals received			Usable lens pairs§
	Number	Sex	Age (days)	
		1965		
1	10	male, female	64–66	10
2	10	male	64–66	10
4	10	male, female	66	10
6	10	male, female	65–67	10
8	10	female	64–68	10
		1966		
30	10	male	70	9
91	13	female	70	9
123	13	male	71	8
199	13	male	70	8
215	11	male	70	9
245	14	male	71	13
273	10	female	71	8
306	10	male	70	9
339	11	female	69	9
393	13	male	67	6

* In weeks for 1965, in days for 1966.
§ One lens from each pair fixed, the other processed fresh.

gave information regarding the variation between the eyes of individual animals, but they were not used in analyzing the effect of fixation time (and the specimens are omitted in Table 9) because specific controls were lacking.

The lenses were removed from the fresh eyes as soon after death as possible. The maximum time lapse was 20 minutes, and most lenses were removed in less than 10 minutes. After removal and blotting, the lens was immediately placed in a shell vial and weighed. Readings were made to the nearest 0.1 milligram. The vial and lens were then ovendried for 18 hours at 120° C. After drying, the vial and lens were removed, cooled 20 minutes at room temperature, and reweighed. The lenses of the eyes placed in formalin were removed at the end of their designated fixation period and processed in the same manner as the fresh eyes. All lens removal, drying and weighing were done in an airconditioned room having a relatively constant temperature of about 74° F. and a relative humidity of 22 per cent.

Weights of fixed and fresh lenses differed for each fixation period (Table 10). In all cases mean wet weights were less for the fixed lenses than for the fresh lenses for a given period. Dry weights

TABLE 10. STATISTICAL EVALUATION OF CHANGES IN WEIGHT IN RELATION TO PERIOD OF FIXATION IN FORMALIN FOR EYE LENSES FROM ALBINO RATS

Period of fixation§	Mean lens weight (milligrams)		Change (per cent)	Degrees of freedom	Value of *t*
	Control†	Treatment‡			
Wet weight — 1965					
1	31.1	29.8	− 4.4	9	3.33**
2	31.7	29.9	− 6.0	9	3.16*
4	31.3	28.9	− 8.3	9	3.43**
6	30.4	27.8	− 9.4	9	2.63*
8	31.0	26.8	−15.7	9	9.33**
Dry weight — 1965					
1	12.7	12.9	+1.6	9	0.69
2	12.9	13.0	+0.8	9	0.36
4	12.7	13.4	+5.5	9	1.94
6	12.2	12.8	+4.9	9	4.62**
8	12.9	13.2	+3.2	9	2.22*
Wet weight — 1966					
30	34.2	30.0	−14.0	7	6.18**
91	32.8	28.6	−14.7	8	4.16**
123	35.4	32.1	−10.3	7	3.98**
199	32.9	28.6	−15.0	8	5.44**
215	32.3	28.0	−15.4	8	12.65**
245	32.2	28.0	−15.0	12	7.12**
273	32.2	28.5	−13.0	7	5.36**
306	32.7	28.6	−14.3	8	6.03**
339	30.8	27.3	−12.8	8	9.72**
393	32.6	28.6	−14.0	5	11.76**
Dry weight — 1966					
30	13.7	14.3	+4.4	8	4.29**
91	13.3	13.7	+3.0	8	2.50*
123	14.1	14.7	+4.3	7	2.61*
199	13.3	13.9	+4.5	7	2.73*
215	13.3	13.9	+4.5	8	3.53**
245	13.2	13.8	+4.5	12	2.50*
273	13.1	13.5	+3.0	7	1.00
306	13.2	13.8	+4.5	8	2.22*
339	12.9	13.8	+7.0	8	4.29**
393	13.1	14.1	+7.6	5	5.00**

* Significant at 5 per cent level.
** Significant at 1 per cent level.
§ In weeks for 1965, in days for 1966.
† Lenses processed fresh.
‡ Lenses processed after fixation in formalin.

exhibited the reverse relationship. A possible explanation of this apparent paradox involves osmotic exchange of water. The lens water is slowly drawn out as a lens becomes fixed, resulting in lowered weight. But the fixation process renders the lens less permeable, and, when it is dried, the net amount of moisture driven off is less than that for a fresh lens.

The trend in weight relationships is evident when the data for the two years are considered collectively. The degree of difference in wet weight between fixed and fresh lenses increased for approximately 6 weeks and then remained relatively constant. When tested by a standard *t*-test (Snedecor, 1957), the differences were found to be significant at the 1 per cent level. Differences in dry weight between fixed and fresh lenses were smaller than those in wet weight.

Figure 15. Change in weight in relation to period of fixation in formalin for eye lenses from albino rats, as represented by departure (in per cent) of mean weight of fixed lenses from that of unfixed lenses at successive intervals. (Values for wet weight actually negative)

Again using the *t*-test, they were significant at either the 5 per cent or 1 per cent level from approximately 4 weeks on. The group fixed for 273 days was a lone unexplained exception. The pattern in each case is shown in Figure 15.

It is apparent that fixation time had a pronounced effect on lens weight. This effect was most noticeable in wet weights as evidenced by the substantial increase in the difference between fresh and fixed lenses during the first 6 weeks. Although relatively constant after that, the difference was significant throughout the maximum fixation period of 393 days. For dry weights significant differences between fresh and fixed lenses did not occur until after approximately 4

weeks of fixation. This difference was relatively constant until after about 48 weeks when a sharp increase was noted which presisted throughout the remainder of the study period.

In interpreting lens data, failure to recognize the effect of fixation time could result in overestimating age on the basis of dry weight and in underestimating it on the basis of wet weight. For dry weight, standardization of a fixation period of 2 weeks or less or one of between 4 and 40 weeks appears desirable. It does not appear that dry weights from these two periods would be directly comparable.

For fish Parker (1963) noted that the amount of initial change in body weight may largely depend upon the state of osmoregulation or amount of body water at the time of original measurement. He further stated that this initial reaction and the subsequent partial return toward original weight undoubtedly reflect chemical changes brought about by the preservative, which alter the osmotic strengths of fluids within the body and within the cells. He showed that significant weight changes are associated with length of time in formalin and with formalin diluted with water of different ionic strengths. He found that, in formalin diluted with fresh water, fish gained weight rapidly for one or two days and then lost weight at a decelerating rate to the time of last measure at 225 days. Initial gains to from 116 to 127 per cent of the original mean weight were noted, followed by declines to from 105 to 111 per cent of the original weight. On the other hand, fish stored in formalin diluted with salt water lost weight for the first few days and then gained at a decelerating rate. Initial weight fell to from 87 to 91 per cent, but subsequently rose to from 91 to 95 per cent, of the original weight. In both instances, the larger relative changes were associated with the smaller fish.

To the extent that they are comparable, the results of this study closely parallel Parker's findings for fish. But the relationship between size and initial weight change cannot be determined from these data because all the specimens were of approximately equal age (64-71 days). In this respect, however, it seems likely that lenses from juvenile animals would be affected more than those from adults since the moisture content is greater in the juveniles. In other studies the author has found the water content of juvenile rat lenses to approach 65 per cent while that of adults was approximately 55 per cent.

FREEZING AND DECOMPOSITION

Collection of eye lenses under usual field conditions may involve a time lapse varying from several hours a day or more before the

specimen can be processed. During this time, they may be subjected to below-freezing temperatures, relatively warm temperatures, or combinations of both. For this reason, the effects of freezing or decomposition on lens weight must be understood in order to interpret data from such specimens. To study this factor, field conditions were simulated. Lenses were subjected to below-freezing, cool, and relatively warm temperatures for various lengths of time. Comparisons were made for fixed lenses as well as fresh lenses.

Known-age guinea pigs and albino rats being sacrificed for other purposes were used. Groups of approximately 10 animals each were subjected to different treatments. The heads from these animals were removed at time of death and immediately assigned to a test group and processed accordingly.

For guinea pigs the groups and treatments were as follows:

A. Refrigerate overnight, then hold at room temperature for 2 hours.

B. Refrigerate overnight, place outdoors in sun for 4 hours, refrigerate a second night, then hold at room temperature for 2 hours.

C. Refrigerate overnight, then place outdoors in sun for 3 hours.

D. Freeze overnight, then hold at room temperature for 4 hours.

E. Freeze overnight, place outdoors in sun for 4 hours, freeze a second night, then hold at room temperature for 4 hours.

F. Freeze overnight, then place outdoors in sun for 3 hours.

G. Hold in trunk of car overnight, then place outdoors in sun for 5 hours.

Each animal furnished a control eye and a treatment eye. The control eye was removed as soon after the death of the animal as possible and immediately processed. But the rest of the head was subjected to the assigned treatment before the treatment eye was removed. Otherwise, however, both eyes for a given specimen were processed in the same way. Eyes of Groups A through F were processed without fixation, and both wet and dry weights of the lenses were measured. Those of Group G were fixed in 10 per cent formalin for 30 days prior to lens removal after which both wet and dry weights of the lenses were measured. Ovendrying time was 19 hours at 130° C. Other temperatures involved were as follows: freezer, —10° C.; refrigerator, +4° C.; maximum outdoor air temperature, +30° C.

(86° F.); minimum outdoor air temperature, +10° C. (50° F.); average room temperature, +21° C. (70° F.).

For rats the groups and treatments were as follows:

A. Refrigerate for 18 hours.

B. Refrigerate for 18 hours, then hold at room temperature for 6 hours.

C. Refrigerate for 18 hours, hold at room temperature for 6 hours, refrigerate for 18 hours, then hold at room temperature for 6 hours.

D. Freeze for 18 hours.

E. Freeze for 18 hours, then hold at room temperature for 6 hours.

F. Freeze for 18 hours, hold at room temperature for 6 hours, freeze for 18 hours, then hold at room temperature for 6 hours.

As with the guinea pigs, the control eye was removed as soon after death as possible while the treatment eye was not removed until completion of the treatment. All eyes were fixed in 10 per cent formalin for 7 days immediately after being removed from the head. Only dry weights were measured. It was necessary to expose the heads of Group D at room temperature for 45 minutes before they thawed enough for the eyes to be removed. Lenses were dried for 45 hours at 80° C. Other temperatures were as follows: refrigerator, +4° C.; freezer, −15° C.; room temperature, +28° to +33° C. (82° — 91° F.).

Data for the guinea pig specimens (except Group G) are given in Table 11. For wet weights, the treatment lenses were heavier than the control lenses for Groups A, B and C that were refrigerated but not frozen before being allowed to thaw for several hours; however, they were lighter for Groups D, E and F that were frozen before similar thawing. The differences were highly significant for all except Group A. After ovendrying, the treatment lenses in Groups A, B and C remained heavier, and those in Groups D, E and F lighter, than the corresponding controls, but the differences were much less and were highly significant only for Groups E and F.

Nearly similar tests on lenses of albino rats resulted in dry weight differences that were significant for the specimens in Group E which had been frozen and thawed at room temperature prior to fixation in formalin (Table 12). When this treatment was carried through another complete cycle (Group F), the difference rose from 5.4 to 13.3 per cent of the weight of the control lenses and was

Table 11. Effects of Freezing and Thawing on Weight of Fresh Eye Lenses from Adult Guinea Pigs

Treatment§	Mean lens weight (milligrams)		Change (per cent)	Standard deviation		Standard error of estimate
	Control	Treatment		Control	Treatment	
			Wet weight			
A	64.9	66.4	+ 2.3	3.17	2.81	1.01
B	66.9	72.9	+ 9.0	3.83	4.74	2.96**
C	65.5	68.3	+ 4.3	3.32	2.06	2.69**
D	63.3	50.7	−19.9	1.94	6.75	4.77**
E	66.9	58.5	−12.6	2.72	6.47	2.68**
F	65.5	49.5	−24.4	3.32	5.90	5.93**
			Dry weight			
A	22.8	23.3	+ 2.2	0.99	0.71	1.16
B	23.2	24.6	+ 6.0	1.14	2.68	1.44
C	22.8	23.8	+ 4.4	1.04	0.96	2.32*
D	22.0	21.1	− 4.1	0.59	1.48	1.50
E	23.4	19.1	−18.4	0.87	3.03	3.07**
F	22.8	20.4	−10.5	1.04	1.64	3.20**

* Significant at 5 per cent level.
** Significant at 1 per cent level.
§ See text.

Table 12. Effects of Freezing and Thawing on Weight of Formalin-fixed, Ovendried Eye Lenses from Adult Albino Rats

Treatment§	Mean lens weight (milligrams)		Change (per cent)	Standard deviation		Standard error of estimate
	Control	Treatment		Control	Treatment	
A	24.0	24.1	+ 0.4	0.78	0.92	0.25
B	20.8	20.9	+ 0.5	0.69	0.74	0.31
C	27.9	27.8	− 0.4	0.98	0.71	0.26
D	22.7	22.3	− 1.8	1.40	2.10	0.42
E	20.4	19.3	− 5.4	0.87	1.26	2.24*
F	24.8	21.5	−13.3	1.06	1.18	6.47**

* Significant at 5 per cent level.
** Significant at 1 per cent level.
§ See text.

highly significant. For both groups the treatment lenses weighed less than the controls.

Guinea pig lenses that were subjected to decomposition for approximately 24 hours before fixation (Group G) averaged 5.3

milligrams less in wet weight than the controls, a difference of 8.3 per cent. This difference was highly significant. Ovendrying these same lenses removed most of the difference, however, and the remaining 0.1 milligram differential between control and treatment lenses was not significant.

From these data it is evident that post mortem changes occurred within the eyeball, and the type of change was influenced by external factors. In general, whether measured by wet weight or dry weight, lenses that were not frozen gained weight in relation to their control counterparts, while lenses that were frozen lost weight.

Scammon and Hesdorffer (1937) noted that a lens begins to absorb water from the surrounding tissues within a short time after death. This absorption would result in increased lens weight. Possibly this accounts for the increases observed for treatments where freezing was not a factor. The amount of or rate at which water is absorbed by a lens was not investigated during this study, but it was found that weight gain occurred in proportion to length of time between death and weighing.

During periods of decomposition it is likely that water absorption by a lens would be accelerated. At the same time, however, breakdown of the tissues would be taking place which would probably result in losses of fluids and cellular materials. This second reaction would gradually destroy the ability of a lens to maintain fluids and result in weight losses.

Montgomery (1963) found significant weight losses to occur in the dried lenses of juvenile racoons that had been exposed to decomposition for more than two days before fixation in formalin. While no significant difference was noted during this study for dry weights of guinea pig lenses that had been subjected to decomposition for 24 hours before being fixed, the highly significant loss observed for wet weights indicates a more fluid state of the lens than normal. In such an instance, fixation in formalin would result in water being drawn out of the lens and into solution, thus reducing weight. At the same time, fixation would cause a lens to become less permeable, and ovendrying would result in less moisture being driven off during a given period of time than in the case of a fresh lens. It is probable that, had decomposition time been extended, dry weights also would have shown losses.

Since much of the lens and its surrounding fluids consists of water, freezing can be easily accomplished. If this were done soon after the death of the animal, the post mortem absorption of water by the lens would be avoided.

Montgomery (1963) found that lenses from juvenile and adult raccoons were reduced in weight by being frozen for more than one

day before fixing and drying. Longhurst (1964), however, did not find freezing for 4 days to have any effect on the weights of sheep lens. Only fixed lenses that had been subjected to combinations of freezing and decomposition lost weight in this study. Longhurst removed the eyes from his specimens before freezing them while Montgomery did not. Similarly, eyes were not removed prior to freezing in this study. It was necessary to let the heads thaw before the eye could be removed and placed in formalin. Some decomposition of eye tissues may have taken place during this time and also during the period after death before freezing was complete.

It is likely that the combined effects of decomposition and freezing cause mechanical damage to the lens capsule and cells as a result of the contraction and expansion of tissues during these processes. The presence of debris in the ocular fluids of lenses subjected to these treatments lends credence to this theory.

Both freezing and decomposition greatly increase the difficulty of working with unfixed lenses. The eye becomes less firm and more difficult to handle. Gross changes are evident in the texture and appearance of the ocular fluids, and considerable debris is noted in these fluids. Also, the lens capsule is more easily ruptured while the lens is being removed. Such rupture results in substantial loss with respect to either wet or dry weight.

CONCLUSIONS

Studies have indicated for several species that eye-lens weight increases as an animal grows older. In the present study this was demonstrated for white rats. For wildlife species, the technique affords a possible means of determining the age composition of a population when other methods are inadequate or lacking.

For white rats, curves for both wet and dry weight, and for fresh as well as formalin-fixed specimens, give equally usable indices. Measurements of specific gravity also reflect weight changes. The data permit more than a simple juvenile vs. adult separation.

In the light of present knowledge, fixing the eye in formalin, followed by lens removal, ovendrying and weighing, is the preferred method. The length of time in formalin must be considered when evaluating data obtained in this way. Under field conditions, quick freezing may be used to preserve lenses until they can be placed in formalin or processed further. But eyes so treated should not be allowed to thaw between freezing and fixing.

There is need for further study to standardize the technique and to determine its applicability to other species.

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RELATIONSHIP BETWEEN EYE-LENS WEIGHT AND VARIATIONS IN DIET[1]

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ABSTRACT

No significant differences were found among eye-lens weights from Wistar-strain laboratory rats maintained from weaning (21 days) until 10 weeks of age on diets of different nutritional levels. Test groups included rats fed three-quarter and one-half rations and others fed rations reduced in protein, energy, Vitamin A content or combinations of them. Rats fed a standard diet were used for comparison.

Body weight responses to the treatment diets were most pronounced in male rats. Final body weights of male rats fed diets reduced in protein, energy or both were significantly lower than those of the controls; for females, only those fed diets reduced in energy showed similar responses. No significant weight differences occured between the control groups and rats fed the Vitamin A-free diet.

Statistical analysis showed no correlation between eye lens weight and body weight for rats of similar age and sex.

Investigations of the eye lens as a criterion of the age of mammals have suggested that nutrition may influence lens weight (Lord, 1962; Edwards, 1962; Friend and Linhart, 1964). If it does, it would have to be considered when using lens weights for age determination in analyzing the composition of wild populations. The present study was undertaken to test the hypothesis that lens growth is affected by the nutritional status of the animal. It was carried on from October 1964 to March 1965. Domestic rats were used to facilitate the experimental design. Extrapolation to other species, whether wild or domestic, would require further research.

The work was divided into two phases: (1) to determine the effect of partial starvation on lens growth; (2) to determine the effect of nutritionally different diets on lens growth.

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MATERIALS AND METHODS

The physical facilities were provided at Griffin Laboratory of the New York State Department of Health. Albino rats of the Wistar strain were used. They were all obtained at weaning age (21 days) and the full compliment for each of the two phases of the work was received at the start of that phase.

All cages were designed to prevent the rats from eating their feces. The floors were of 14 gauge galvanized wire with a 2 x 2 mesh (½-inch openings). Feces dropped through the mesh to removable trays or drawers below. Cages were cleaned weekly by removing and washing these trays or drawers.

Powdered diets were used throughout the study. Food offered was measured daily to the nearest 0.1 grams on a Harvard trip balance and placed in individual feeding units. A feeding unit consisted of a 2-ounce jar, a 1½-inch square of 14-gauge hardware cloth with ½-inch mesh, and a 53-millimeter cap with a 1¼-inch hole punched in it. A rat obtained food by inserting his head through the hole in the cap and pressing down on the hardware cloth which had been placed on top of the food. Pressure against the food would cause it to pass through the mesh and become available to the rat. Unconsumed food was removed each day and weighed. All food containers were washed a minimium of twice each week, and oftener if necessary, to prevent uneaten food from caking in the jars.

Identification of individual rats, cages, food storage containers and feeding unit jars was maintained by differential marking and tagging.

At the completion of each phase, the animals were killed by ether asphyxiation. Both eyes were then removed within 15 to 30 minutes after death; the lens from one was immediately processed and the other eye was placed in 6.5 milliliters of freshly mixed 10 per cent formalin for 7 days. Lenses were removed according to procedures outlined by Friend (1967).

Lenses were ovendried in glass shell vials for 18 hours at 120° C. and then cooled for 20 minutes at room temperature, after which they were weighed to the nearest 0.1 milligram on a direct-reading balance. The individual vials and lenses were placed in the drying oven in groups of 10 at staggered intervals to allow for the time required for weighing, so that the drying time and the interval between drying and weighing were constant for all samples.

All processing of lenses was done in an air-conditioned room. Relative humidity and room temperature were measured with a sling psychrometer at hourly intervals throughout the weighing

periods and were found to be reasonably constant (25 to 28 per cent and 73° to 76° F., respectively).

Data for food consumption were recorded daily for each rat on standard forms. All animals were weighed every seven days on a spring balance, and weights were recorded to the nearest gram. Lens weights were read to the nearest 0.1 milligram, relative humidity to the nearest whole per cent, and room temperature to the nearest degree Fahrenheit.

PHASE I—QUANTITATIVE STUDY

During this phase 42 rats were used to test the effects of partial starvation on lens weight. They were separated by sex, weighed and placed in categories of under 35, 35 to 40, and over 40 grams. The numbers in each category were 10, 15 and 17, respectively. They were divided into control and treatment groups (Table 1) in

Table 1. Weight Distribution of Laboratory Rats in Control and Treatment Groups at Start of Phase I

Group	Body weight in grams				Mean weight (grams)
	Under 35	35–40	Over 40	Total	
Control	2	4	4	10	39.4
¾ ration	4	6	6	16	38.1
½ ration	4	5	7	16	38.6
Total	10	15	17	42	38.5

which there were equal numbers of each sex. The animals were then reweighed, and the mean weights for the groups were found to be satisfactorily similar. All the animals were held in one rack containing 36 single-compartment cages to which six additional single cages were added. A mixed distribution of the three groups among these cages was maintained.

Feeding

A single diet in powder form was formulated to meet the basic requirements for normal growth in the rat as reported by Farris and Griffith (1949) and Warner (1962). Its composition is given in Table 2. Each animal was fed the same amount for the first three days, receiving 15 grams on the first day and 10 grams on the third day; they ate little the first day and were not fed on the second. Average food consumption was 8 grams per rat per day.

TABLE 2. COMPOSITION OF DIET USED IN PHASE I

Component	Per cent
Vitamin-free test casein	18.00
D, L methionine	0.23
Corn oil	5.00
Vitamin mixture	2.20
Jones-Foster salt mixture	4.00
Carbohydrate mixture*	70.57

*Consisted of: cornstarch, 60 per cent; sucrose, 20 per cent; dextrose, 20 per cent.

This value was used as a starting point and the control group was offered 8 grams on the fourth day, while the two treatment groups received 6 and 4 grams, respectively. For the remainder of this phase the average amount per animal consumed by the control group during a given 24-hour period determined the amount offered all rats the following day. Control rats were then offered 2 grams more than they ate the preceding day, and the two treatment groups were given three-quarters and one-half of what the controls had eaten. During the first 24 days of differential feeding both sexes were treated alike, but during the remaining 16 days consumption data were calculated separately by sex and feed was offered accordingly.

During the first 34 days of differential feeding, both sexes were offered the same amount of food. It was then recognized that the control males were consuming more than the females which resulted

TABLE 3. AVERAGE FOOD CONSUMPTION (IN GRAMS) OF CONTROL GROUP IN PHASE I

Food	October 5–November 7	November 8–23	Total
	Male		
Amount offered*	431.0	251.0	682.0
Amount eaten	382.6	236.1	618.7
Percentage eaten	89	94	92
	Female		
Amount offered*	431.0	207.8	638.8
Amount eaten	356.5	186.4	542.9
Percentage eaten	83	90	85

*Prior to November 8 all rats were fed equal amounts daily. After that the males were offered slightly greater amounts than the females.

in the females in the two treatment groups receiving slightly more than their planned proportions. This was corrected during the remaining 16 days by determining the food consumption of the control males and females separately. Nevertheless, the control males consumed a higher percentage of the food offered them than did the females even when the females were offered less than the males (Table 3). Animals in both treatment groups consumed nearly all the food offered throughout the study.

Effect on Body Weight

During this phase of the experiment increasingly wide variations in body weight occurred among the rats in each treatment group and of both sexes (Figure 1). Analysis of variance for the final weights indicated that these differences were highly significant for both males and females ($F = 166.55$ and 30.32, respectively, with

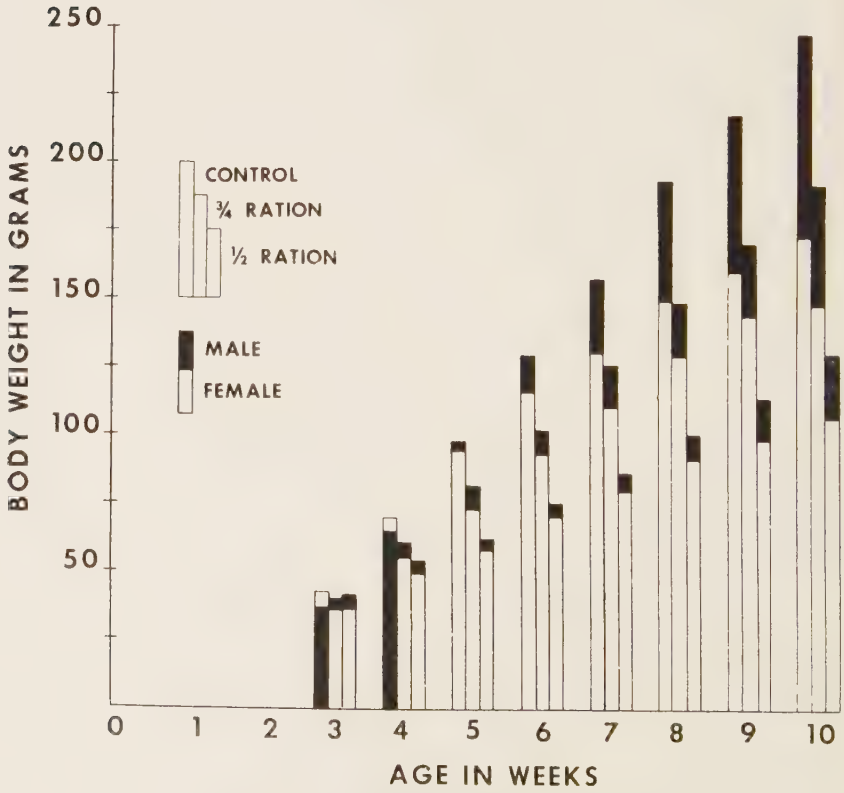

Figure 1. Weekly increase in body weight for male and female albino rats in the three groups studied during Phase I.

2 and 18 degrees of freedom). While significant variations from the controls occurred in both treatment groups, the magnitude of the variations was not the same for the two sexes. Differences in final body weight between the controls and the treatment groups were most pronounced in male rats. Males fed three-quarter and one-half rations weighed 22.8 and 47.6 per cent less, respectively, than the corresponding controls, while females weighed 14.5 and 37.2 per cent less, respectively.

Mean weekly body weights (Table 4) were tested statistically by analysis of covariance (Table 5), taking into consideration both sex and treatment. The results must be interpreted with caution since severity of treatment was found to decrease the amount of variance around the regression lines. Because of this, a simple analysis of covariance apparently is not wholly adequate for testing the data statistically. However, from the data presented in Table 4, the following biological observations were made.

For males:

1. At 3 weeks of age no meaningful differences in body weight occurred between any of the groups.
2. At 4 weeks, both the control group and the group on three-quarter rations were heavier than the one on one-half rations. This condition continued throughout the remainder of the study.
3. At 5 weeks, the control group was noticeably heavier than all other groups and remained thus throughout the study.

For Females:

4. At 3 weeks of age the control group was heavier than either of the two treatment groups (both of which had similar weights).
2. No meaningful differences occurred between the two treatment groups at 3 weeks.
3. At 4 weeks, the control group was heavier than either treatment group, and the group on three-quarter rations was heavier than the one on one-half rations.
4. At 8 weeks, a slight reduction in the degree of difference in weight between the control group and the one on three-quarter rations was noticeable. This reduction persisted throughout the remainder of the study period, but the weight differences still remained.

Table 4. Weekly Mean Body Weights (in grams) for Males and Females in Each Group During Phase I

Group	Number of rats	Age in weeks							
		3	4	5	6	7	8	9	10
Male									
Control	5	36.6	66.0	98.0	128.6	157.0	192.4	217.0	246.0
¾ ration	8	40.0	61.0	80.9	101.5	124.9	148.1	168.8	190.0
½ ration	8	41.0	53.9	62.0	74.7	86.4	99.7	113.2	129.2
Female									
Control	5	42.2	69.8	94.2	115.6	130.4	148.0	158.8	172.4
¾ ration	8	36.1	55.2	74.1	93.0	109.9	127.6	137.8	147.0
½ ration	8	36.2	49.4	58.4	70.4	80.2	91.1	98.1	105.8

Table 5. Analyses of Covariance of Body Weight for Males and Females in Each Group During Phase I§

Group or statistic	Regression coefficient (logarithms)	Deviations from regression		
		Degrees of freedom	Sum of squares of deviations	Mean square
Male				
Control	404.7205	38	6,664.0	175.4
¾ ration	277.4897	62	13,651.5	220.2
½ ration	163.5559	62	4,120.1	66.4
Within groups	..	162	24,435.6	150.8
Regression coefficient	..	2	47,114.7	23,557.4
Common regression	845.7661	164	71,550.3	436.3
Female				
Control	251.7039	38	6,570.6	172.9
¾ ration	211.3496	62	10,895.9	175.7
½ ration	127.3592	62	7,573.0	122.1
Within groups	..	162	25,039.5	154.6
Regression coefficient	..	2	12,319.2	6,159.6
Common regression	590.4127	164	37,358.7	227.8

§ $F = 156.22^{**}$ for males and 39.84^{**} for females with 3,162 degrees of freedom.

There was little doubt that the treatment diets had a pronounced effect on the growth rate of the rats being studied.

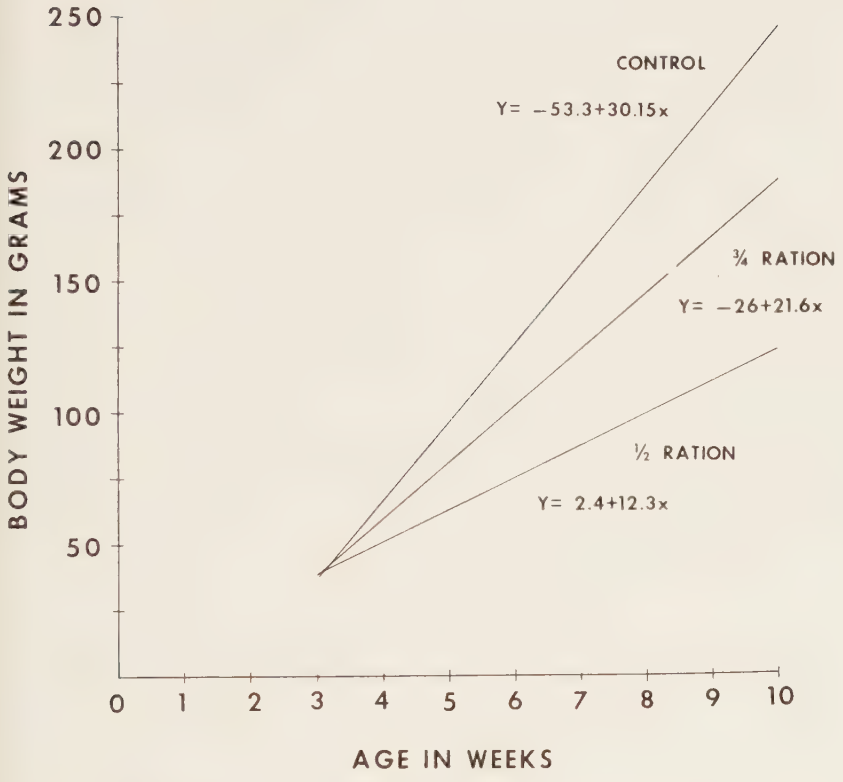

Figure 2. Regression in body weight according to diet for male albino rats during Phase I.

As a further check, growth rate regressions were determined for each group (Figures 2 and 3). For males the relationship between body weight (y) and age (x) was linear for each group during the entire study period and was described by the equation $\hat{Y} = \bar{y} + b(X - \bar{x})$. This relationship did not appear to describe the data for females, however.

Females in all groups gained weight in a linear fashion through 7 weeks of age, but then a slowing down and gradual leveling off occurred. To describe these data in terms similar to those for the males, the values for age were transposed into logarithms. These data were then described by the foregoing equation and clearly showed that, if the normal food requirement at weaning was reduced by one-quarter or one-half, body growth in female rats was significantly reduced. The more severe the reduction in nutrient intake, the slower the growth rate as shown by the following values for the regression coefficient (b): 251.7 for the control group, 211.3 for

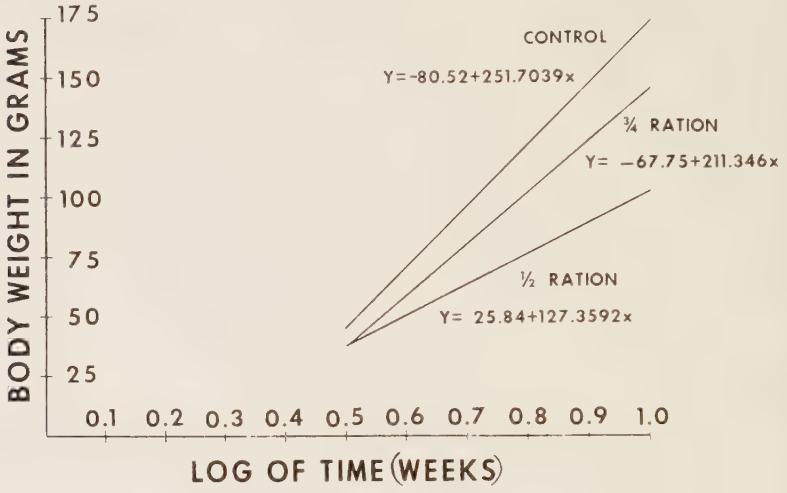

Figure 3. Regression in body weight according to diet for female albino rats during Phase I.

the three-quarter ration group, and 127.4 for the one-half ration group. Males grew at a more rapid rate for a longer period of time and were more severely affected by reduction in food during this period, and values for b were 30.15, 21.60 and 12.3, respectively.

Effect on Lens Weight

Lenses were processed by formalin fixation and ovendrying as described by Lord (1959). Lens weights for the group on one-half rations were generally slightly lower than those for either the control group or the one on three-quarter rations. The lack of response of lens growth to partial starvation is shown in Table 6. Of interest is the low variation associated with the group on one-half rations, the most severe treatment.

Table 6. Eye Lens Weights for Males and Females in Each Group at End of Feeding Period in Phase I

Group	Male		Female	
	Number	Weight*	Number	Weight*
Control	5	13.8 ± 0.28	5	13.4 ± 0.17
¾ ration	8	13.8 ± 0.13	8	13.4 ± 0.20
½ ration	8	13.6 ± 0.09	8	13.2 ± 0.13

* Mean weight in milligrams (± standard error).

Analysis of variance for the lens weights for all groups indicated no significant differences within groups of either males or females ($F = 0.74$ and 0.48, respectively, with 2 and 18 degrees of freedom). Again using analysis of variance, data for all males combined tested against those for all females, and the lenses from males were found to be heavier to a highly significant degree ($F = 8.28$ with 1 and 40 degrees of freedom).

PHASE II—QUALITATIVE STUDY

During this phase 160 rats were used to test the effects of variations in diet on lens weight. They were weighed initially and placed in weight categories of under 30, 30 to 39, 40 to 50, and over 50 grams. Equal numbers from each category were then selected until 10 of each sex had been placed in a control group and in each of seven treatment groups. The animals were reweighed and the average body weights for the several groups were found to be relatively uniform (Table 7).

The number of animals used required more than one rack of cages. All animals were confined individually in cages similar to those described under Phase I, the 80 male rats being confined in one large double rack in the center of the room, while the 80 females were placed in four racks laterally located and facing the males. A mixed distribution of all groups was maintained in each rack.

Feeding

During this phase a control diet and seven modifications were used. The composition of each is given in Table 8. The modified diets were reduced in energy, protein, vitamin A and combinations of these factors. Alphaceal, a non-nutritive filler, was used to replace components of the diet which were removed to develop the desired nutritional deficiencies.

A feeding schedule, in which the weight of food offered was gradually increased, was constructed from the data obtained during Phase I. The daily consumption figures for the control group in Phase I were used as a base, but they were slightly reduced in an effort to obtain maximum consumption by the control group in Phase II. The weight of food offered all eight groups was the same for any given day, but when Alphaceal was used as a filler it caused considerable variations in volume. Males received more food than females throughout this phase.

Animals on energy-deficient diets consumed a higher proportion (98 to 99 per cent) of the food offered than did other groups

Table 7. Weight Distribution of Laboratory Rats in Control and Treatment Groups at Start of Phase II

Sex	Body weight in grams					Mean weight (grams)	
	Under 30	30–39	40–50	Over 50	Total	Each sex	Both sexes
Control							
Male	3	3	2	2	10	38.9	39.3
Female	2	3	3	2	10	39.7	
½ energy content							
Male	3	3	2	2	10	38.1	39.4
Female	2	3	3	2	10	40.7	
½ protein content							
Male	3	3	2	2	10	38.3	39.7
Female	2	3	3	2	10	41.1	
½ energy and ½ protein content							
Male	3	3	2	2	10	38.1	39.1
Female	2	3	3	2	10	40.1	
Vitamin A-free							
Male	3	3	2	2	10	38.8	39.6
Female	2	3	3	2	10	40.4	
Vitamin A-free and ½ energy content							
Male	3	3	2	2	10	39.5	39.3
Female	2	3	3	2	10	39.1	
Vitamin A-free and ½ protein content							
Male	3	3	2	2	10	39.4	39.3
Female	2	3	3	2	10	39.2	
Vitamin A-free and ½ energy and ½ protein content							
Male	3	3	2	2	10	39.6	39.6
Female	2	3	3	2	10	39.5	

(90 to 95 per cent) as shown in Table 9. Also, individual variation in food consumed was lowest for those on the energy-deficient diets.

TABLE 8. COMPOSITION OF DIETS USED IN PHASE II

Diet	Designation*	Percentage of each component							
		Vitamin-free test casein	D, L methionine	Corn oil	Vitamin mixture	Jones-Foster salt mixture	Carbohydrate mixture§	Alphacel†	Total
Control	A	18.0	0.230	5.0	2.2	4.0	70.57	0	100.0
½ energy content	B	18.0	0.230	5.0	2.2	4.0	19.61	50.96	100.0
½ protein content	C	9.0	0.115	5.0	2.2	4.0	79.68	0	100.0
½ energy and ½ protein content	D	9.0	0.115	5.0	2.2	4.0	28.72	50.96	100.0
Vitamin A-free	E	18.0	0.230	5.0	2.2‡	4.0	70.57	0	100.0
Vitamin A-free and ½ energy content	F	18.0	0.230	5.0	2.2‡	4.0	19.61	50.96	100.0
Vitamin A-free and ½ protein content	G	9.0	0.115	5.0	2.2‡	4.0	79.68	0	100.0
Vitamin A-free and ½ energy and ½ protein content	H	9.0	0.115	5.0	2.2‡	4.0	28.72	50.96	100.0

*As used to designate groups in Tables 9, 10, 11, 12, 13, and 14.
§Consisted of : cornstarch, 60 per cent; sucrose, 20 per cent; dextrose, 20 per cent.
†Non-nutritive bulk filler (also a carbohydrate).
‡Vitamin A-free.

TABLE 9. FOOD CONSUMPTION OF CONTROL AND TREATMENT GROUPS DURING PHASE II*

Group	Male				Female			
	Number	Food			Number	Food		
		Offered (grams)	Eaten			Offered (grams)	Eaten	
			Grams	Percent			Grams	Percent
Vitamin A-supplemented diets								
A	8	4947	4704	95.1	8	4392	3934	89.6
B	8	4947	4927	99.6	7	3294	3244	98.5
C	8	4947	4456	90.1	7	3843	3644	94.8
D	9	5566	5520	99.2	9	4941	4919	99.6
Vitamin A-free diets								
E	10	6184	5766	93.2	9	4941	4560	92.3
F	7	4329	4309	99.5	9	4941	4887	98.9
G	8	4947	4522	91.4	9	4941	4514	91.4
H	8	4947	4868	98.4	9	4941	4867	98.5

* Includes only animals that survived the entire experiment.

Effect on Body Weight

There was some mortality among the experimental animals during this phase. As losses occurred, unequal distributions of body-weight classes among the treatment groups also occurred, resulting in unequal base lines for comparisons. Therefore, it was necessary to devise a method of adjusting body weights within groups to allow valid comparisons throughout the study. This was done in the following manner:

1. The groups were originally made up of 10 individuals each and had comparable weight distributions.
2. When an animal died in one group, the animal of the same sex in each of the other groups, whose initial body weight was closest to that of the rat lost, was then disregarded in tabulating weight data for the reminder of the study.
3. As a result, weight data for the first week (3 weeks of age) were based on 10 animals in each group, and those for the final week (10 weeks of age) was based on five animals in each group.

As in Phase I, increasingly wide variations in body weight occurred among the rats in each treatment group and of both sexes as the study progressed (Figures 4 and 5). Analysis of variance for the final weights showed that these differences were highly significant for both males and females ($F = 28.18$ and 14.15, respec-

Table 10. Relative Loss in Body Weight for Groups Fed Different Diets in Phase II

Group	Loss in body weight*	
	Male	Female
Vitamin A-supplemented diets		
B	45.8	42.2
C	21.5	2.9
D	48.6	49.1
E	0.4	0.6
Vitamin A-free diets		
F	47.8	49.1
G	14.3	4.0
H	55.0	47.4

* As percentage of body weight of control group.

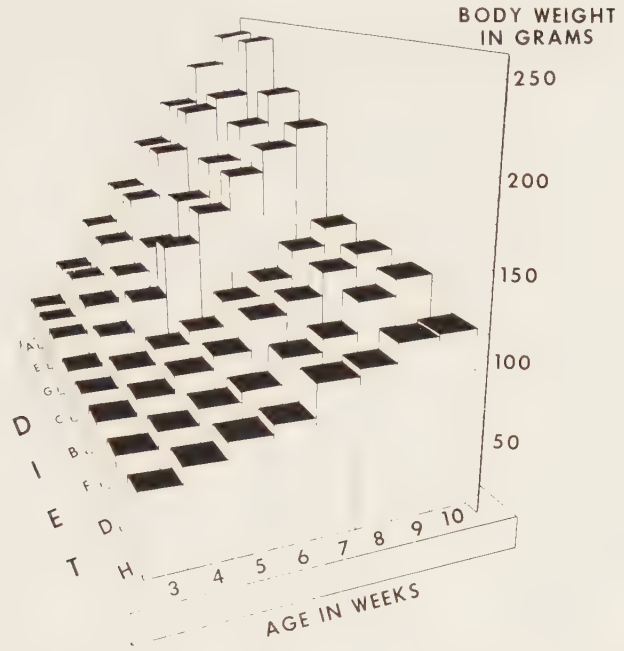

Figure 4. Relative weekly body weights of male albino rats according to diet during Phase II.

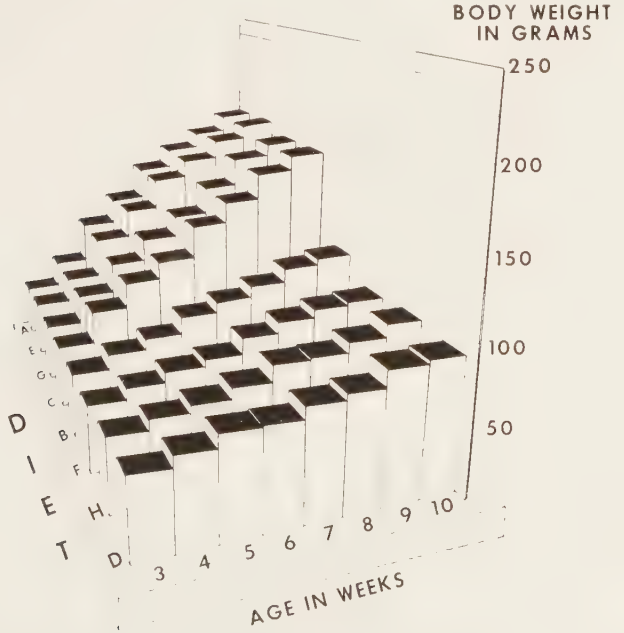

Figure 5. Relative weekly body weights of female albino rats according to diet during Phase II.

Table 11. Weekly Mean Body Weights (in grams) for Males and Females in Each Group During Phase II

Group	Age in weeks							
	3	4	5	6	7	8	9	10
Male								
Vitamin A-supplemented								
A	38.9	69.0	105.1	135.6	169.0	199.3	228.4	250.8
B	38.1	53.6	64.6	74.4	92.0	103.0	122.6	136.0
C	38.3	60.9	84.0	110.0	144.0	170.2	184.8	197.2
D	38.1	51.0	62.0	72.4	89.0	97.5	116.8	129.0
Vitamin A-free								
E	38.8	70.8	104.4	136.7	172.2	202.7	209.8	250.2
F	39.5	52.7	64.2	75.0	96.0	105.2	121.8	130.6
G	39.4	62.2	89.0	110.1	142.7	168.7	195.2	214.6
H	39.6	52.7	64.8	71.7	92.3	98.2	110.8	112.8
Number in each group	10	9	8	7	6	6	5	5
Female								
Vitamin A-supplemented								
A	39.7	61.3	90.3	105.6	133.6	149.0	160.6	172.8
B	40.7	52.3	60.8	73.0	82.8	89.2	98.6	102.8
C	41.1	66.1	84.8	99.0	123.2	138.4	154.6	167.8
D	40.1	50.3	58.6	60.1	69.0	73.2	83.8	87.8
Vitamin A-free								
E	40.4	65.7	91.4	113.6	136.0	149.0	161.8	172.2
F	39.1	48.1	56.4	60.6	72.6	79.2	85.4	87.8
G	39.2	62.8	85.4	101.6	117.0	137.2	157.0	166.4
H	39.5	49.3	55.6	61.7	73.6	75.4	83.2	91.4
Number in each group	10	7	7	7	5	5		5

tively, with 7 and 32 degrees of freedom). Differential effects were also noted between sexes. Final mean weights of control males were significantly greater than those of all other males, with the exception of one group which was fed a vitamin A-free diet. Males fed diets reduced in energy content were 46 to 55 per cent lighter than the controls; in contrast, those fed protein-deficient diets were 14 to 21 per cent lighter. Final mean weights of females fed energy-deficient diets were 42 to 49 per cent lower than the controls; in contrast, those fed diets reduced only in protein content were 3 to 4 per cent lighter. (Table 10).

Mean weekly weights were considered by sex and treatment (Table 11). The control groups were tested statistically against the treatment groups by analysis of covariance (Table 12). Severity of treatment was again found to decrease the amount of variance around the regression lines. The following conclusions were drawn

TABLE 12. ANALYSES OF COVARIANCE OF BODY WEIGHT FOR MALES AND FEMALES IN CERTAIN GROUPS DURING PHASE II§

Group or statistic	Regression coefficient (logarithms)	Deviations from regression		
		Degrees of freedom	Sum of squares of deviations	Mean square
Male				
A	404.7259	54	24,960.4	462.2
C	316.6760	54	17,830.6	330.2
B	175.7567	54	6,848.5	126.8
H	145.5421	54	12,206.4	226.0
Within groups	.....	216	61,845.9	286.3
Regression coefficient	.....	3	74,478.4	826.1
Common regression	1,042.7007	219	136,324.3	622.5
Female				
A	260.3458	49	30,813.6	628.8
C	238.3524	49	17,432.4	355.8
B	120.6197	49	10,562.7	215.6
H	94.1825	49	5,567.4	113.6
Within groups	.....	196	64,376.1	328.4
Regression coefficient	.....	3	51,892.4	297.5
Common regression	713.5004	199	98,268.5	493.8

§$F = 86.71^{**}$ with 3,216 degrees of freedom for males and 52.67^{**} with 3,196 degrees of freedom for females.

from the biological characteristics of the data rather than from statistical analysis.

1. At 3 weeks of age no meaningful difference in body weight occurred between any of the groups.
2. From 5 weeks on, all rats fed energy-reduced diets were lighter than the corresponding controls.
3. From 5 weeks on, all male rats fed protein-reduced diets were lighter than the corresponding controls, but females on the same diets continued to gain weight at only slightly lower than normal rates.
4. All rats fed the vitamin A-free diet gained weight at a rate comparable to that of the controls throughout the study period.

Body weights were altered by all but one of the treatment diets. It was also noted that the rate of gain was not uniform between the sexes. For the control groups, weekly weights were plotted for each animal that survived the entire study period (Figure 6). These data showed that generally the weight of a rat at 10 weeks was determined by its weight at 3 weeks.

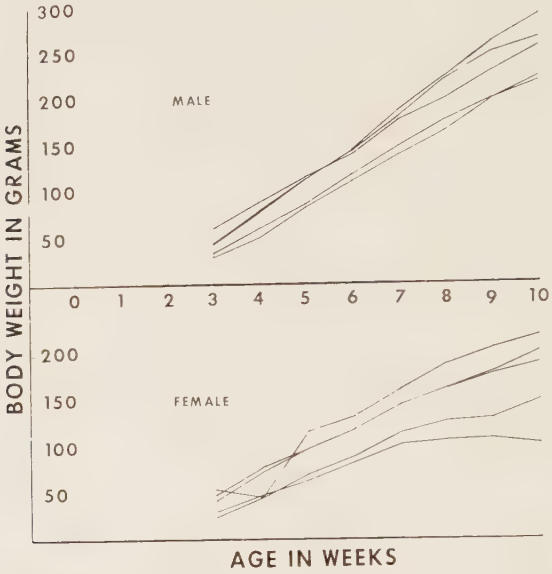

Figure 6. Individual growth patterns for albino rats in the control groups which survived the entire study period during Phase II.

To determine the actual slope of these data for the various diets involved, growth rate regressions were calculated. These regressions were calculated only for the control group and for three treatment groups: one-half energy content; one-half protein content; and the combination of vitamin A-free, one-half energy content and one-half protein content. Statistical analysis showed that the vitamin A-free diet did not alter body growth (Table 10), and therefore these regressions were felt to describe all the data.

Body weights for all groups, except for the control females, increased linearly throughout the study period and are described by the equation $\hat{Y} = \bar{y} + b\ (X - \bar{x})$. For the control females, weight was plotted against $\log_{10}$ of age (Figure 7).

To compare the control males and females, the weights for males were also plotted against $\log_{10}$ of age. Mean weights for the males were greater, to a highly significant degree, then those for the females; values for the regression coefficient (b) were 404.7259 and 260.3458, respectively. Among the males, weight data for all three treatment groups had regression slopes significantly lower than that of the control group; values for b were 31.14 for the control group, 24.36 for the one-half protein group, 13.78 for the one-half energy group, and 11.14 for the combination diet group. Regressions for females in the treatment groups indicated that those fed

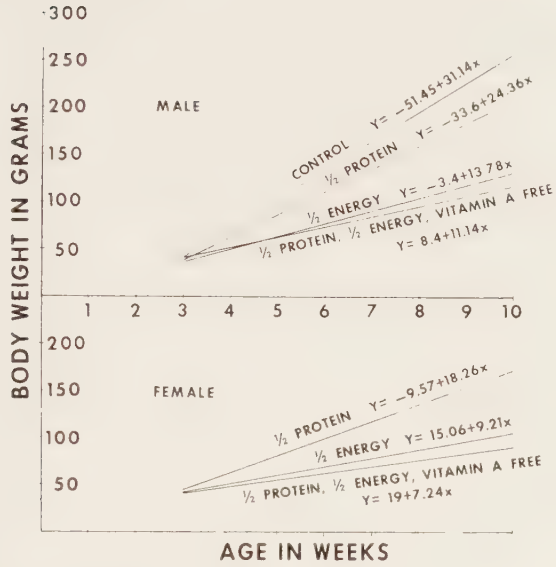

Figure 7. Regression in body weight according to diet for male and female albino rats during Phase II.

diets reduced only in protein content grew more rapidly than those fed diets deficient in energy (Figure 7). The slope for the rats fed the protein-deficent diet was approximately twice that for either two treatment groups; values for b were 18.26 compared with 9.21 and 7.24, respectively.

The data show clearly that males in the control group and in each of the treatment groups grew more rapidly than the corresponding females. The relationship between the slopes of the growth rates for the two sexes was relatively constant for the control group, the one-half energy group and the combination diet group. Female

TABLE 13. RATE OF GAIN IN BODY WEIGHT FOR GROUPS FED DIFFERENT DIETS IN PHASE II

Group	Value of b*		Ratio of female to male (per cent)
	Male	Female	
A	404.7259	260.3458	64
C	316.6760	238.3524	75
B	175.7567	120.6197	69
H	145.5421	94.1825	65

* In logarithms (see text).

rats on protein-deficient diets were proportionately heavier, however (Table 13).

Weight-regression data were compared weekly by sex and the following conclusions were drawn.

1. Control males were slightly lighter than females at 3 weeks of age, slightly heavier at 4 weeks, and heavier to a highly significant degree at 6 weeks.
2. Males on the one-half energy diet followed a pattern similar to that of the controls except that the point at which weights of the two sexes became significantly different was delayed 3 weeks.
3. Males on the one-half protein diet overtook females at 6 weeks of age and became significantily heavier at 8 weeks, but the difference was not significant at 10 weeks.
4. Growth of both male and female rats on a diet deficient in energy, protein and vitamin A was erratic.

Effect on Lens Weight

Body weights as shown in Table 11 were adjusted as has been described to permit valid comparisons between all treatments despite losses among the experimental animals. Although, for purposes of body-weight analysis, the number of animals considered at the completion of the study had been reduced by half, many of those for which some data were disregarded were still alive. The question then arose as to whether the lens weights for these animals could be used. To answer this, a correlation analysis of body weight and lens weight was made for the control animals. These animals were tested separately by sex, and no correlation was found for either males or females ($r = 0.26$ with 18 degrees of freedom for males and 0.38 with 16 degrees for females.). Therefore, lens weights for all surviving animals were used for analysis since no surviving animals were ever actually removed during the experiment.

As in Phase I, analysis was based on lenses processed by formalin fixation and ovendrying as described by Lord (1959). The data are summarized in Table 14. Lens weights were checked at random throughout this part of the study by reweighing four or five lenses from each drying rack after all initial weighing had been completed. Forty-one of the 133 lenses were reweighed. Of these, 18 weighed exactly the same, six were 0.1 milligram heavier, three 0.2 milligram heavier, ten 0.1 milligram lighter and four 0.2 milligram lighter than when originally weighed.

An analysis of variance of the lens weights for all groups indi-

TABLE 14. EYE LENS WEIGHTS FOR MALES AND FEMALES IN EACH GROUP AT END OF FEEDING PERIOD IN PHASE II

Group	Male		Female	
	Number	Weight*	Number	Weight*
Vitamin A-supplemented diets				
A	8	14.8 ± 0.20	8	13.8 ± 0.32
B	8	14.0 ± 0.21	6	14.1 ± 0.16
C	8	14.6 ± 0.24	8	14.6 ± 0.18
D	9	14.3 ± 0.29	9	13.9 ± 0.25
Vitamin A-free diets				
E	10	14.6 ± 0.26	9	14.2 ± 0.28
F	7	14.1 ± 0.28	9	13.8 ± 0.25
G	8	14.2 ± 0.22	9	14.2 ± 0.29
H	8	14.1 ± 0.17	9	13.8 ± 0.17

* Mean weight in milligrams (± standard error).

cated no significant differences between the control group and any of the treatment groups ($F = 1.36$ with 7 and 59 degrees of freedom for males and $F = 1.24$ with 7 and 60 degrees of freedom for females). Data for all males combined were tested against those for all females, and the lenses from males were found to be significantly heavier ($F = 5.78$ with 1 and 132 degrees of freedom). Further testing showed control males to be the only group significantly heavier than the corresponding females (standard error of the mean differences $=2.70$*).

DISCUSSION

Both phases of this study dealt with the affect of starvation or inanition on growth of the eye lens. Inanition can be in many forms and by definition implies lack of, or non-assimilation of, food.

PHASE I

That the female rats in the two treatment groups were fed a little more than planned during the first 34 days probably induced only slight error since average daily food consumption through most of that time was quite similar for both sexes. Average calculated daily food requirements for rats were given by Warner (1962). According to his data, males require increases of 1 gram at 33 to 35 days of age, 3 grams at 42 to 44 days and 6 grams by 53 days.

During the period when overfeeding occurred in the present study, the animals were 21 to 54 days of age.

The method of pacing used to determine daily food allotments was satisfactory. When the rats were given an abundance of food they ate less than when smaller amounts were offered. For this reason, food quantity was kept at a minimum in an attempt to induce maximum consumption by the greatest number of individuals. Food consumption obtained was comparable to that of one group of Wistar rats maintained on experimental diets by Adams (1964).

The data presented clearly illustrate that the rate of increase in body weight for both male and female rats in the treatment groups was significantly lower than that of the control group because of the reduction in food intake. This result was not unexpected and has been well documented by others for the rat and other animals. Also, differences in final body weight between the control and treatment groups were most pronounced in male rats (Table 4). Similar observations were made by Jackson and Stewart (1920) during their studies of the effects of inanition in young rats. The more rapid rate of growth observed in the male accentuated its response to limited intake.

Compositiion of the diet used (Table 5) was based on the minimum nutrient content necessary to produce desirable growth in the rat. It appears that body weight gains among the control group were below those which could be expected based on the data of Warner (1962) and Adams (1964). Warner pointed out, however, that the curve he constructed exceeded growth rates of the Wistar strain reported elsewhere in the literature. He further noted that it was almost certain that growth data reported by others had not included those for runts or other abnormal animals. Adams used Wistar-strain rats, but included only animals which showed a steady weight gain. In the present study all animals were included irrespective of growth tendencies. While weight gain certainly did not approach the maximum, the weights of the control group are felt to be representative of what might normally be expected for the strain and colony used.

The straight-line presentation of the data should be mentioned. The growth rate of the rat, like that of other animals, is not described by a straight line, but takes the form of a sigmoid curve. Post-weaning growth, however, can be described by a straight line when the logarithm of body weight is plotted against the reciprocal of time in weeks (Zucker *et al.,* 1941).

This experiment was conducted during the period of most accelerated growth in the rat. Visual examination of the 21- to 70-day portion of Warner's growth curve indicates that the relation-

ship between body weight and age is probably linear for the male and curvilinear for the female. When the data from Phase I were examined statistically, these suspected relationships were found to have occurred. Thus, no transformation of the data for males was necessary to describe growth as a straight line, but it was necessary to plot body weight against the $\log_{10}$ of age to obtain such a relationship for females.

The similarity in lens weight for the control rats and those fed the reduced rations was surprising at first. It could be expected that when growth is retarded in an animal from weaning until past the normal age of sexual maturity, to the degree observed during this study, differences in structural size and weight of the various components of the body would also result. Jackson (1915a) and Stewart (1918) investigated the growth differences of the various organs and body parts in relation to variations in diet. They reported that, although growth in body weight in young rats may be completely suppressed by underfeeding for considerable periods, certain organs and parts of the body have a strong tendency to continue growing. They found responses varying from loss in weight to increase in weight for the various organs and parts of the body studied in rats underfed from weaning until 10 to 12 weeks of age.

The eye is one of the organs which continues to grow during underfeeding. Falck (1854) and Bitsch (1895) indicated that the absolute weight of the eyeball in the dog did not lose weight during inanition, and Sedlmair (1889) reported similar findings for the domestic cat. Among the early reports of similar findings for the rat were those by Jackson (1915a, 1915b, 1925 and 1932), Stewart (1919) and Jackson and Stewart (1920).

Slight losses in eyeball weight have been recorded under certain circumstances. Jackson (1937) reported that eye weights of young male rats subjected to a 15-week period of underfeeding followed by a return to normal rations were lower than those of control animals. Kudo (1921) recorded average losses in eyeball weight of 10.2, 13.3 and 13.0 per cent during various water-inanition tests with rats.

While these observations were made on the eyeball rather than on the lens itself, they are undoubtedly valid indications of lens growth under similar conditions. The validity of this hypothesis is suggested by the findings of Norrby (1958) who reported that the lens and eyeball grew at the same rate from 16 to 543 days in 345 rats. He also reported that the increase in volume of the lens and eyeball were positively correlated during the entire growth period. These observations indicate that, normally, lens growth is not reduced or retarded in lower animals by quantitative inanition.

Haiti (1913) observed that, in general, weights of the organs of rats closely follow body weight and, therefore, may be considered a function of body weight. If this were valid for the eye lens, a correlation should exist between body weight and lens weight. No such relationship was found during this study, indicating further the independent nature of lens growth.

While body weight is a good general measure of growth in animals, it is not necessarily a good indicator of the growth of certain component parts of the animal or of age. McMeekan (1940) in his work with pigs noted the inadequancy of live weight as a measure of growth throughout the animal body and pointed out that the growth of the whole is the resultant of differential growth of the constituent parts. When this growth is prevented by underfeeding, growth does not cease in all parts (Jackson, 1932). The eye is one of these parts. During total inanition there is little or no loss in the weight of the eyeballs in lower animals; body weights decrease, but eyeball weights continue to increase in young animals and either increase at a lower rate or decrease slightly in adults (Jackson, 1925).

Phase II

Since only the food offered could be controlled, it was necessary to feed minimum daily amounts to obtain comparable consumption by all animals. Maximum consumption by the control groups was desired but not obtained. Control males consumed a higher proportion of the food offered than did all other groups except those on energy-deficient diets. Control females, however, consumed the lowest proportion.

Of the treatment groups those on protein-deficient diets (with full energy compliment) consumed the least amount of food. This is an agreement with the findings of others. Black *et al* (1950) showed that reduced food intake is a characteristic behavior for rats on low-protein diets. Rats on energy-deficient diets consumed the highest proportion of the food offered as was expected.

The relative differences in food consumption by the various treatment groups were not felt to have any significant effect on body weight. On the average, male control rats in Phase II consumed 31 grams less food, and female 51 grams less, than did the corresponding animals in Phase I. Despite these differences, the males in Phase II weighed 34 grams more, and the females 14 grams more, at 10 weeks of age than did their Phase I counterparts (Table 15).

It was recognized, despite careful measuring of components and hand mixing of all diets, that the 50 per cent reduction desired

TABLE 15. COMPARISON OF FOOD CONSUMPTION AND BODY WEIGHT FOR CONTROL GROUPS IN PHASES I AND II

Item	Male		Female	
	Phase I	Phase II	Phase I	Phase II
Food consumption*				
Amount offered	682	618	639	549
Amount eaten	619	588	543	492
Body weight*				
At start	37	39	42	40
At end	217	251	129	173

* In grams.

TABLE 16. ANALYSIS OF DIETS FED DURING PHASE II

Diet	Analysis						
	Moisture	Protein	Fat	Fiber	Ash	Nitrogen free extract	Vitamin A*
Vitamin A-supplemented							
A	6.31	15.7	5.01	0.32	3.65	69.01	5.9§
B	5.93	16.2	4.87	29.50	3.66	39.84	11.4
C	6.54	8.7	3.88	0.44	3.68	76.76	16.8
D	6.53	8.7	4.28	30.10	3.45	46.94	14.7
Vitamin A-free							
E	5.99	16.4	4.63	0.34	3.66	68.98	4.5§
F	6.39	17.6	4.83	29.54	3.84	37.80	0.0
G	5.78	8.6	4.26	0.35	3.40	77.61	0.0
H	5.54	8.8	4.65	31.47	3.53	46.01	0.0

* Expressed as units per gram.
§ Indicates possible error in formulating this diet at source (see text).

in energy and protein content of the various test diets would not be precisely obtained. Therefore, all diets were analyzed chemically to determine what each treatment group actually received (Table 16). The New Jersey Feed Laboratory determined moisture, protein, fat, fiber, ash, carbohydrate and nitrogen free extract content for each diet. Vitamin A determinations were provided by the feed testing laboratory at the University of Massachusetts.

The striking differences exhibited in body weight between the

various treatment groups illustrate the effects of variations in diet on body growth. From these data and calculated regression lines for body weight (Table 13) two questions arise: why did the protein-deficient diets have a greater effect on males than on females; and why was no measurable effect produced by the vitamin A-free diet?

Proximate analysis of the experimental diets disclosed an error which probably occurred when the diets were mixed. The control diet contained less than the desired amount of vitamin A, while the vitamin A-free diet contained vitamin A at a level only slightly lower than the control diet. All other diets were as ordered. This error was not discovered until after the period of experimental feeding. Data from the three diets actually containing no vitamin A did not provide a good measure of the effects of this deficiency because of the interactions of other deficiencies present in them.

The data do not show that the vitamin A-free diet limited growth, and thus these data cannot be used to infer the effects of vitamin A on eye lens weight. Undoubtedly the time period involved was too short for the stores present in the animals before the experimental treatment to be depleted. The rats were not put on a vitamin A-deficient diet before being placed on the experimental diets, and the newborn rats probably had received enough vitamin A from the mother to build a sufficient store to carry them through the relatively short experiment period.

McLaren (1963) reported that the first symptom of vitamin A deficiency was a slowing down of the growth rate. In young rats he found that the earliest signs appeared after 6 weeks of deficiency. A similar time period was given by Warner (1962). Rats in the present study were maintained on a vitamin A-free diet for only 6½ weeks. There is some evidence that a deficiency was starting to occur when the study was terminated. Final body weights of control rats were slightly greater than those of the vitamin A-free rats. These differences were not significant but represented a reversal from previous weeks. This change was greatest in males, and this agrees with the literature.

Mayer and Krehl (1948) reported that male rats seemed unable to check a drop in vitamin A, while females were capable of retaining their reserves of this vitamin much longer. Similar observations had been made earlier by Coward (1942), and lower body weights of rats on vitamin A-deficient diets were reported by Patterson *et al.* (1942).

Interpretation of results is further complicated by the interactions which result between vitamin A and other deficiencies. Rats on diets deficient only in vitamin A normally become sick and die after 8 weeks on such a diet (McLaren, 1963). When other

deficiencies are added, however, or low levels of vitamin A are fed, life expectancy may be measurably increased (Paul, 1946).

McLaren (1963) and others have reported that the rate of growth is closely related to the expenditure of vitamin A. Vitamin A increases the efficiency of the food of rats in maintaining or producing tissue growth (McLaren, 1959). Rats deprived of this vitamin and also restricted in growth by other inadequacies of diet use up their vitamin A stores at a less rapid rate than those fed diets reduced only in vitamin A. In such animals, symptoms of vitamin A deficiency are noticeably delayed.

Other workers have commented on the effects of protein-deficient diets on body weight in the rat. Limson and Jackson (1932) did not find any differences between the sexes resulting from low-protein diets among the rats they studied, while Jackson (1936) and McLaren (1963) both reported proportionately greater weight losses among male rats on low-protein diets. As noted previously, reduced food consumption occurred in low-protein diets, and in this study males fed such diets consumed proportionately less food than did females (Table 9).

Body weights for the control group in this phase compared more favorably with those reported in the literature than did those recorded during Phase I. All rats used were Wistar strain but were received from two different sources. Variations in the growth rates reflected strain and colony differences since both were fed the same diet; the animals used in Phase I consumed higher quantities (Table 15).

The relationship between body weight and age for the time period studied was found to be linear for males and curvilinear for females during Phase I. During Phase II, this relationship was linear for treatment females, but for all other groups the pattern was the same as during Phase I (Figure 7). The change in pattern for treatment females is felt to have been the result of influences asserted by the protein and energy deficiencies of the diet. No explanation for this is apparent.

Various workers had previously indicated that eye weight and lens weight might be influenced by nutrition. Hatai (1913) listed nutrition and physical condition as one of four factors modifying the relative weight of the organs, and Clapp (1913) reported slight variations in lens weight corresponding to nutrition. More recently, Donaldson and King (1937) observed that growth changes are mainly correlated with age, but body weight has a slight effect on the weights of the eyeball and the lens. The greater weight of lenses from captive-reared animals compared with those from wild-trapped animals, as reported by Lord (1959, 1962), has already been noted.

Dietary deficiencies in energy, protein and vitamin A had no apparent effect on lens weight in this study (Table 14). The data appear to be conclusive and are supported by other reports in the literature. McLaren (1963) observed that slight retardation of lens and eye growth resulted in rats on a low-protein diet for 50 days, but in other studies he failed to find any changes in the lens attributable to diet among rats on a similar low-protein diet. Limson and Jackson (1932) found significant increases in weight for the eyes of rats fed a low-protein diet from weaning to 4 months of age.

Dische *et al.* (1956) studied the changes in lens proteins of rats during aging and reported that the amount of protein increases during the whole life span of the animal. This growth apparently continues in the face of severe dietary deficiencies as reported by Addis *et al.* (1963) who found no loss of protein from the eyes of albino rats during a 7-day fast.

McLaren (1963) observed that vitamin A has not been isolated from the lens and that a deficiency of it is not known to produce any changes in the eye. Specifically, he noted that the lens is not affected by a vitamin A deficiency.

It is interesting that among all the animal groups used in the present study, lens weight for males was significantly greater than that for females only in the control groups (14.8 vs. 13.8 milligrams). Two possible explanations are offered: (1) in the males lens growth may have been retarded by the treatment employed, while in the females it was not affected; or (2) the control group may not have been typical.

The more rapid rate of increase in body weight among males and the more severe reaction of males to various types of inanition illustrate that in the rat a sex differential exists regarding the response of body weight to inadequacies in diet. This response may apply to lens growth also. From the data presented in Table 14 it may be seen that lens weight for control males exceeded those for all other groups, while those for control females were either no greater or less than those for all other groups. The distribution of these weights shows that, for the males, both the greatest proportion falling in the heaviest weight classification (15 milligrams) and the lowest proportion falling in the lightest weight classification (13 milligrams) were from the control groups. These data might be interpreted as supporting the hypothesis of differential sex response.

A second explanation for the significant difference in lens weight between control males and females is the possibly skewed distribution of the weights for the females. This explanation appears more logical. There were more females than males represented in the lowest category (12 milligrams), and the converse was true

for the higher weight categories. Also, the greatest variance in lens weight (0.82) occurred among the control females (Table 14). Had distribution in the control group been typical, and had diet affected lens growth among the males but not among the females in the treatment groups, there should have been a similar distribution of lens weights among the treatment females. Such a distribution is not evident, however.

Lending further support to this explanation is the distribution of lens weights within the vitamin A-free group. This diet was eliminated as a source of growth deficiency for the animals studied, and these animals may be considered as an additional control group. When lens weights for females in this group were compared with weights for those in the control group, considerable differences were found. Approximately 34 per cent of the lenses for the vitamin A-free group weighed less than 14 milligrams, while the proportion was approximately 62 per cent for the control group. The distribution for the vitamin A-free group was more typical of other treatment groups.

CONCLUSIONS

Quantitative reduction in rations of the same diet, for laboratory rats from weaning (21 days) until the age of 10 weeks, results in slower growth as measured by body weight. Differences are more pronounced in males than females. But such reduction in nutrition has no significant effect on eye lens weight, although the lenses of males average heavier than those of females.

Qualitative alterations in diet also reduce the rate of gain in body weight, and to a greater degree in males than females, but do not significantly affect eye lens weight. Reduction in the energy component has a greater effect than reduction in the protein component. The effect of a vitamin A deficiency on lens weight was not determined because the experimental period was too short for vitamin A stores to be fully depleted. Female rats are able to maintain nearly normal body weight on diets containing only half the normal protein requirement, but males cannot. Increased nutritional deficiency lessens the variance among both body weights and eye lens weights.

There is no correlation between eye lens weight and body weight for laboratory rats of similar age and sex. Eye lens weight is not affected by post-weaning alterations in nutrition and, under these conditions, is a reflecton of age, not nutritional status.

ADDENDUM

It is recognized that the study reported here represents only

a beginning in the evaluation of the eye lens as a criterion of growth and related characteristcs in mammals. Since this work was completed, data have been obtained from white-tailed deer in New York which indicate that nutrition does have an effect on lens growth in deer (Friend and Severinghaus, 1967). This finding, however, does not conflict with those of the present study because the data suggest that the differences observed were a result of poor nutrition prior to weaning or even during fetal development. In the present study lowered levels of nutrition were imposed only after weaning.

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A REVIEW OF RESEARCH CONCERNING EYE-LENS WEIGHT AS A CRITERION OF AGE IN ANIMALS[1]

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ABSTRACT

A review of the pertinent literature concerning growth of the eye lens is presented together with a discussion of variables inherent in evaluating lens-weight data as a criterion of age in wildlife species. The findings of 33 other studies are summarized. The importance and application of the technique in wildlife biology is also discussed.

Walls (1942) stated that "the [eye] lens is unique among the organs of the body in that its development never ceases, while senescence commences even before birth". Lord (1959) showed that data for dry weight of the eye lens can be used to determine the age composition of populations of the cottontail rabbit (*Sylvilagus floridanus*), and numerous other investigators have studied this relationship for a variety of wildlife species. Reports of these investigations are scattered throughout the literature in a variety of journals and other publications. It is the purpose of this paper to summarize this material to the extent it has come to the attention of the author.

The application and meaningful interpretation of data concerning eye lens weight are dependent upon an understanding of the growth of the lens and the factors that influence that growth. With respect to wild animal populations, an understanding is also needed of the variables associated with collection and processing which might influence lens weight.

GROWTH OF THE LENS

Although studies of the eye lens and its possible use as a criterion of age for wildlife species are quite recent, the human eye has been studied for many years. Information concerning laboratory rats and some domestic animals has also been recorded.

Man

The first quantitative observations on the dimensions of the eye are credited to a French ophthalmologist, du Petit, who reported on the weight of the lens about 1720–1730 (Scammon and Wilmer, 1950). The first detailed report on this subject was by Smith (1883)

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who summarized the knowledge on lens growth to that time and quoted the following passages by Otto Becker in the Handbook of Graefe and Saemisch:

> "In the new-born child the lens is smaller than in the adult, and of a more globular form; it is clear as water, very soft, and nearly equally soft in all its layers. The comparison as to size is true, however, only of the equatorial diameter, for according to Jaeger the antero-posterior diameter is equal, or very nearly so, to that of the adult lens. The changes which take place during life are referrable to two opposing processes.
>
> So long as the whole individual grows, that is up till about the 25th year, new lens-fibres are laid down at the equator and apply themselves, as they further develop, to the anterior and posterior surfaces, each fibre bending round the equator. Hence the lens continually increases in its transverse diameter, and gradually loses its spherical form. The addition of the new fibres at the equator has little influence on the thickness of the lens, because it is only when the fibres have attained their maximum length that they reach to the poles. Thus the surface of the lens assumes a flatter curvature and the dioptric power diminishes.
>
> The slight increase in thickness which would nevertheless occur is nullified to a great extent by the peculiar process of sclerosis which affects the fibres of the lens progressively from earliest youth until old age, and which ceases, unless pathological processes intervene, only with the death of the individual.
>
> The sclerosis of the lens-fibres is the analogue of the hardening of the cuticle, only that while in the cuticle the older cells lie on the external surface, in the lens they are constantly driven inwards towards the centre by those more newly formed. In other respects the analogy is complete. The younger lens fibres are thicker, softer, richer in water, freer from colour; the older they grow the more they give up their water and become flat, closely compacted, and ambercoloured. And since the centrally situated fibres are for the most part the oldest, the sclerosis begins at the centre of the lens and progresses thence towards the surface."

To this summary Smith (1883) added one important fact, i.e., growth of the lens does not cease with that of the rest of the body, but is continuous throughout the whole period of life. This is the basic concept upon which use of the eye lens as a criterion of age, as applied to wildlife, is based. He reported this increase to be rather constant and noted that the volume increased in nearly the same proportions as weight. These observations were summarized and commented on by Bowman (1883), but he offered no additional information.

Many others have contributed to the knowledge of eye development. E. T. Collins (1894) was the first to report on the histology of the developing eye. W. J. Collins (1905), noting increases in lens weight for humans from birth to 80 years, stated "alone of all the tissues of the body the lens seems to live an enchanted existence endowed with endless growth". Clapp (1913), working with infants, found the increase in lens weight to be gradual from birth until the fifth month.

The weight of the entire eyeball was found to more than double between birth and maturity with most of the growth taking place during the first five years of postnatal life, but no definite evidence

of puberal increase in eyeball weight was noted (Scammon and Armstrong, 1925). In further studies Scammon and Wilmer (1950) determined that the eyeball increased a little less than threefold between birth and maturity.

Studies on the relationship between lens and eyeball growth by Scammon and Hesdorffer (1937) showed the lens to be a decreasing component of the eyeball during the first two decades of life, after which it became an increasingly larger component since the lens continued to grow while the eyeball ceased growing. They reported lens weight at 75 years of age to be four times that at birth, illustrating the continued growth of this structure.

Laboratory Rat

Lowery (1913) noted that the dry substance of eyeballs of the albino rat (*Rattus norvegicus*) increased from 7.4 per cent at birth to 14.4 per cent at 20 days and to 20.15 per cent at one year. This change was much greater than that found in man.

Hatai (1913), working with albino rats, reported that in general the increase in weight of the organs (including eyeballs) parallels the increase in body weight. He concluded, therefore, that in rats growing normally the weight of the organs might be considered a function of body weight. He noted the following four factors as modifying influences on the relative weight of the organs: nutrition, strain of animal, influence of age and season, and sex difference. Jackson (1913) found that eyeballs reached their largest size at 2 weeks and that weight differences according to sex were pronounced. He noted, also, a low variation between eyeball weights within age groups and only a slight correlation between body weight and eyeball weight. He concluded that growth of the eyeball may be somewhat independent of influences affecting the growth of the body as a whole. Freudenberger (1933) also reported a low variability among eyeball weights within a given age group.

Investigations by Donaldson and King (1937) showed variations in both eyeball and lens weight for different strains of the Norway rat. They found weights were greatest in colony albinos and smallest in the captive grays [wild?]. In both sexes and in all the strains, lens weight increased during the entire study period from birth to 550 days. They suggested that rat lenses grow more rapidly than human lenses and concluded that age is the most important datum in relation to the weight of the eyeball and its parts.

Leopold and Calkins (1951) measured eyes from 205 rats ranging in age from 21 to 1,000 days. They noted a continued increase in over-all size with age, and histological sections showed

that the lens increased markedly in capsule thickness as well as in over-all size. Salzmann (1912) and Bellows (1944) had previously described the thickening of the lens capsule with age in humans.

Using data from 345 rats (16 to 543 days old) Norrby (1958) found that the weight and volume of the lens and eyeball increased along an asymptotic curve. He noted that weights were similar for both eyes and that males had heavier values than females. He also reported that the lens and eyeball grew at the same rate and that the increase in volume of these structures was positively correlated during the entire growth period.

Domestic Animals

In his studies with cattle, Salit (1931) found average increases in lens weight of slightly less than 1 gram between 5-week-old calves and 2-year-old cattle. Further work with cattle by Brown and Evans (1935) indicated that little lens growth took place after six years. They interpreted their results to mean "that the lens grows in an autocatalytic manner and that it ceases to grow after reaching its maximum weight". This viewpoint was also held by Krause (1935) who, based on his findings with pigmented and albino rabbits, concluded "the process of growth of the mammalian lens follows that of a monomolecular (or autocatalytic) reaction. The normal mammalian lens grows towards a maximum and not continuously in the manner in which it has been assumed to develop". He also stated that lens weight is dependent on the animal breed (or stock) and that heredity is an important factor in determining gross lens growth.

Wildlife

Lord (1959) found that weights of formalin-fixed, ovendried lenses could be used to determine age in the cottontail rabbit. Since then, investigations have been undertaken on a variety of wild mammals and birds. These are listed in Table 1 together with brief summaries of the findings reported.

Influence of Associated Variables

Eye-lens weight may be influenced by conditions that govern the growth and physical condition of the animal. Also, the operations of collection and processing may affect the weights recorded.

Nutrition

The effects of nutrition on lens growth have been studied in

Table 1. Summary of Literature Concerning Use of the Eye Lens as a Criterion of Age in Wildlife Species.

Species	Author	Remarks
	Mammals	
Cottontail rabbit (*Sylvilagus floridanus*)	Lord (1959)	Lens weight separated juveniles from adults; determined month of birth for juveniles, year of birth for rabbits older than 1 year.
	Wight and Conaway (1962)	Lens weight separated juveniles from adults, superior to epiphyseal X-ray.
	Edwards (1962)	Lens weight separated juveniles from adults.
	Dudley (1963)	Paper not reviewed (lens weight used to determine population age structure in Michigan).
	Rongstad (1966)	Lens weight separated young of the year from adults; separation of other age groups unlikely.
Swamp rabbit (*Sylvilagus aquaticus*)	Martinson *et al.* (1966)	Lens weight separated juveniles from adults.
Black-tailed jackrabbit (*Lepus californicus*)	Tiemeier and Plenert (1964)	Lens weight accurately determined age of hares raised in captivity up to 650 days — the oldest studied.
European rabbit (*Oryctolagus cuniculus*)	Dudzinski and Mykytowycz (1961)	Lens weight was a reliable indicator of age up to approximately 150 days.
Field hare (sp. – ?)	Rieck (1962)	Paper not reviewed.
Fox squirrel (*Sciurus niger*)	Beale (1962)	Lens weight separated juveniles from adults and 2nd-litter juveniles from 1st-litter juveniles; possibly would separate age groups through 2½ years.
Woodchuck (*Marmota monax*)	Davis (1964)	Lens weight varies linearly with age after first year and is useful to determine chronological age of adults.

Gray fox (*Urocyon cinereoargenteus*)	Lord (1961)	Lens weight and tooth wear were in 78 per cent agreement when used to estimate year of birth, 90 per cent agreement when used to separate juveniles from adults.
Red fox (*Vulpes fulva*)	Friend and Linhart (1964)	Lens weight separated juveniles from adults; were in 100 per cent agreement with closure of epiphysis.
Raccoon (*Procyon lotor*)	Sanderson (1961)	Lens weight determined month of birth for animals under 12 months old; estimated relative age composition among older animals.
Howler monkey (*Alouatta caraya*)	Malinow and Corcoran (1966)	Lens mass increased with age in both sexes.
Long-nosed bandicoot (*Perameles nasuta*) Vulpine phalanger (*Trichosurus vulpecula*)	Kingsmill (1962)	Paper not reviewed.
European wild hog (*Sus scrofa*)	Matschke (1963)	Lens weight was not a reliable indicator of age; but, under controlled conditions, dry weight of lens could be used to determine age of pen-raised animals.
Impala antelope (*Aepyceros melampus*)	Grafton (1965)	Lens weight separated lambs from yearlings; rate of lens growth reflected quality of range.
Pronghorn antelope (*Antilocarpa americana*)	Kolenosky and Miller (1962)	Lens weight in straight-line relationship with age from 5 months to 9½ years; age relatively easy to determine up to 3½ years, more difficult after that due to overlapping of weights.
White-tailed deer (*Odocoileus virginianus*)	Lord (1962)	Lens growth corresponded to ascending age curve through 10 years; very limited data.
	Lueth (1963)	Lens weight separated only fawns reliably.
	Lewis (1964) Lewis and Whitehead (1965)	Lens weight used as index to quality of range.
	Friend and Severinghaus (1967)	Lens weight was significantly lower for animals from poor range than for those from good range; overlapping between age groups was much greater among animals on poor range.

Table 1. *(continued)*

Species	Author	Remarks
Mule deer (*Odocoileus hemionus*)	Nellis (1966)	Use of lens weight for determining age can be perfected for prenatal deer.
Columbian black-tailed deer (*Odocoileus h. columbianus*)	Longhurst (1964)	Lens weight was satisfactory criterion of age through at least 5 years when considering only year class.
Bison (*Bison bison*)	Novakowski (1965)	Lens weight distinguished calves, yearlings and adults.
Fur seal (*Callorhinus ursinus*)	Bauer *et al.* (1964)	Lens weight increased geometrically in both sexes and to the age of the oldest specimens studied (a 14-year male and a 21-year female); when only lens weight and sex are known, age can be determined to nearest year through age 2.
	Birds	
House sparrow (*Passer domesticus*)	Payne (1961)	Use of lens weight was unsatisfactory; cited other unsuccessful results for other species (ring-necked pheasant, redwinged blackbird and scaled quail).
Chukar partridge (*Alectoris graeca*)	Campbell and Tomlinson (1962)	Lens weight separated juveniles from adults; unsatisfactory for separating age groups among adults.
Bobwhite quail (*Colinus virginianus*)	Roseberry and Verts (1963)	Lens weight correlated with age, but degree of overlapping too great to permit determining age of individual birds; correlation can be used in comparative studies between different populations.
Sharp-tailed grouse (*Pedioecetes phasianellus*)	Dahlgren *et al.* (1964)	Lens weight separated juveniles from adults; little, if any, increase in weight after the first year.
Ring-necked pheasant (*Phasianus colchicus*)	Dahlgren *et al.* (1965)	Lens weight separated juveniles from adults; little, if any, increase in weight after the first year.

depth by medical researchers whose investigations have been focused on a variety of laboratory animals as well as man. Investigators working with wildlife species have only recently begun to evaluate the influence of nutrition on lens weight. Reports from these two fields of research appear to contradict one another, but in reality they do not.

It was shown quite early that the eye is one of the organs of the body which continues to grow during underfeeding. Falck (1854) and Bitsch (1895) indicated that the absolute weight of the eyeball in the dog did not lose weight during inanition. Similar findings were reported for the domestic cat by Sedlmair (1899) and for the rat by Jackson (1915a; 1915b; 1925; 1932), Stewart (1919) and Jackson and Stewart (1920).

Slight losses in eyeball weights have been recorded under certain circumstances. Jackson (1937) reported that eye weights of young male rats that were underfed for 15 weeks and then returned to normal rations were lower than those of controls. Kudo (1921) reported average losses in eyeball weight of 10.2, 13.3 and 13.0 per cent during various water inanition tests with rats.

While these observations were made on the eyeball rather than on the lens itself, they probably apply to lens growth under similar conditions. This is suggested by the findings of Norrby (1958) who reported that in 345 rats the lens and eyeball grew at the same rate from 16 to 543 days of age. He also reported that increases in volume of the lens and eyeball were positively correlated during the entire growth period. As a whole, these observations indicate that, normally, lens growth in lower animals is not reduced or retarded by quantitative inanition.

The qualitative aspect of nutrition has also been investigated. Hatai (1913) listed nutrition and physical condition as one of four factors modifying the relative weight of the organs. Clapp (1913) reported slight variations in lens weight corresponding to changes in nutrition. Donaldson and King (1937) observed that growth changes are mainly correlated with age, but that body weight has a slight effect on the weight of the eyeball and the lens.

Limson and Jackson (1932) investigated low protein diets in rats and found significant increases in the weight of the eyes from weaning to 4 months of age. Protein deficiencies were also investigated in this species by Addis *et al.* (1936) who found no loss of protein from the eyes during a 7-day fast. McLaren (1963) observed that slight retardation of lens and eye growth resulted in rats on a low-protein diet for 50 days, but in other studies he failed to find any lens changes attributable to diet among rats on a

similar low-protein diet. Friend (1967b) also failed to find any reduction in lens weight among rats on low-protein diets.

Vitamin A deficiency has been investigated, and McLaren (1963) observed that vitamin A has not been isloated from the lens and that a deficiency of it is not known to produce any changes in the eye. Specifically, he noted that the lens is not affected by a vitamin A deficiency.

Several investigations pertaining to wildlife have suggested that nutrition may play an important role in the growth of the lens: Lord (1959) and Edwards (1962) for the cottontail rabbit; Lord (1962), Lewis (1964), Lewis and Whithead (1965) and Friend and Severinghaus (1967) for the white-tailed deer; Friend and Linhart (1964) for the red fox; and Grafton (1965) for the imapala antelope. Matschke (1963) stated that differences in lens weight among penned European wild hogs maintained on different diets was really due to the relationship of eye-lens weight to body weight and not to diet except as diet affected body weight.

The lack of response of lens weight to variations in diet noted in the medical literature appears to contradict the observations reported by wildlife investigators. However, it has been noted by Friend and Severinghaus (1967) that, whereas laboratory studies have involved only progeny from presumably well nourished females, data for wild populations may apply to animals conditioned by nutritional deficiencies affecting the parent females.

Other Variables

Montgomery (1963) found that lenses from juvenile and adult raccoons were reduced in weight by being frozen for more than 1 day before fixing and drying. Longhurst (1964), however, did not find freezing for 4 days to have any effect on the weight of sheep lenses. Similar negative effects of freezing on lens weight for white-tailed deer were found by Friend (unpub.).

Both Montgomery (1963) and Friend (1967a) found decomposition to cause significant changes in lens weight. In addition, Friend found the length of time in formalin prior to drying and weighing to influence lens weight.

IMPORTANCE AND APPLICATION

The importance of techniques for determining age for wild species was emphasized by Taber (1963) who stated "One of the most powerful tools of the wildlife investigator is an understanding of the structure of the population and of the kill". To obtain this information the investigator must use many techniques of age identi-

fication. These techniques vary in sensitivity from a simple juvenile-adult separation (Hale, 1949) to more complex year-class separations (Severinghaus, 1949). Use of the eye lens falls in the latter category, and it is important because it permits the separation of more age groups than has previously been possible with other techniques.

The mammalian lens offers a possible means of determining the age of an animal because of its continuous growth. The biologist measuring this continuous growth and interpreting the resulting data in terms of age groupings must be able to evaluate the influence of variables present. For the wildlife investigator, this evaluation encompasses a broader scope than that required of the laboratory technician working under closely controlled conditions.

Use of lens weight for determining age in wildlife species has much to offer the game biologist. The accuracy and usefulness of this technique, however, is directly correlated with the degree to which the variables present can be properly evaluated. Probably, no single variable causes sufficient change in lens weight to be of major concern, but in combination such factors as the genetic and nutritional background of the animal, sex, time between death and collection, method of collection, method of preservation, time of preservation and method of processing may greatly influence the actual lens weight or that recorded for specimens collected. Once the influence or lack of influence of these and other possible causes of variation are understood and standardized, and uniform methods of preserving and processing lenses are practiced, the technique will become an increasingly important tool.

The inconsistent findings reported in the literature reflect some species differences in lens growth. The continued growth of the human lens has been established beyond any reasonable doubt, but Weale (1963) reported that the human lens differs significantly from other mammalian lenses by a factor which is yet undiscovered.

Lansing (1952) stated that the mammalian lens grows continuously throughout life but that the growth rate is not constant and becomes slower and slower, approximating, but never reaching zero as age advances. Weale (1963) reported that animal lenses cease their growth at a relatively young age. This is in agreement with the work of Krause (1935) with rabbits and of Brown and Evans (1935) with cattle, but it is contradictory to the observations of Donaldson and King (1937) for rats. Lens growth of wild mammals, for the species which have been studied, more nearly conforms to the findings of Krause and of Brown and Evans.

Despite the early leveling off of the lens-growth curve for most wild species, lens-weight data constitute a valuable tool for the wildlife investigator. For some species of mammals they make it

possible to separate litters as well as some age groups among adults where previously only a juvenile-adult age classification could be made (e.g., fox, squirrels, hares and rabbits). Application of this new knowledge may result in the development of additional techniques for judging age through an increased number of known-age individuals in samples collected. Also, use of lens weights as an indicator of the condition of game range may deserve further consideration.

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INFLUENCE OF NUTRITION ON EYE-LENS WEIGHT IN DEER[1]

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ABSTRACT

Eye-lens weights for deer taken during January on three wintering areas in New York were compared. Range conditions were considered good on two of the areas but poor on the other. Lens weights for the two areas of good range were similar, but those for all age groups sampled on the area of poor range were significantly lower. Studies of laboratory rats from well nourished mothers have shown that growth in body weight after the age of weaning can be completely suppressed by underfeeding for considerable periods with no retardation in eye-lens growth. Therefore, it seems probably that the differences recorded for wild deer were the result of nutritional deficiencies that affected lens growth of fawns during their fetal period or prior to weaning. Thus, they are considered to reflect differences in range quality.

Lord (1962) suggested that eye-lens weight might be an index of nutritional status in deer (*Odocoileus*). Other indications that lens growth in wild mammals is influenced by diet are found in the work of Edwards (1962) for the cottontail rabbit (*Sylvilagus floridanus*), Lueth (1963) for the white-tailed deer (*Odocoileus virginianus*) and Friend and Linhart (1964) for the red fox (*Vulpes fulva*). Longhurst (1964) attempted to evaluate the effects of nutrition on lens weight for the Columbian black-tailed deer (*Odocoileus hemionus columbianus*) but could not draw any clear cut conclusions.

This paper compares eye-lens weights for white-tailed deer from three wintering areas in New York that differ in many respects including their carrying capacity for deer. The specimens used were collected during special post-season hunts in January 1965.

DESCRIPTION OF AREAS

Locations of the three areas are shown in Figure 1, and their general characteristics are compared in Table 1.

Ripley Area

This area of nearly 17,000 acres in extreme western New York is part of a narrow flat agricultural belt along the shore of Lake Erie. It consisted largely of orchards and vineyards interspersed with farm woodlots. Deer moved into this area during winter to escape the heavy snows and colder temperatures of the adjacent highlands,

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Figure 1. Location of the three areas from which data were obtained.

but at other seasons they scattered out over an area of some 450 square miles.

Food for deer was plentiful and of good quality. Although no specific studies have been made, mortality from starvation, or attributed to weakness caused by inadequate nutrition, was not recorded during cursory inspections by landowners, local residents and game biologists. Deer killed during winter by accident (cars, trains, dogs, etc.) were in good physical condition.

Seneca Army Depot

This 9,872-acre tract is located a few miles east of Seneca Lake in the central part of the State. The area was once farmed, but since 1942 it has been under the control of the U. S. Army. At that time a 7-foot fence was constructed around the perimeter of the Depot, and the enclosed deer population increased rapidly (Hesselton *et al.*, 1965). Heavy starvation losses occurred during the winters of 1956–57 and 1957–58. Management was begun in 1957, and by 1962 the population had been reduced to near the carrying capacity of the winter range. At the time of this study, deer from this area compared favorably with the best in the State on the basis of reproductive rate, body weight and antler development.

Table 1. Comparison of the Three Deer Wintering Areas from Which Eye-lens Specimens Were Obtained.

Area	Bedrock*	Soil association and type§	Primeval forest type†	Average annual minimum temperature‡ (degrees F.)	Growing season (days)	Condition of winter range
Ripley	Shumla siltstone	Fulton-Toledo silt loam and silty clay loam	Central hardwoods	0 to 5	160–180	good
Seneca Army Depot	Shale and thin limestone	Darien-Romulus silt loam and silty clay loam	Central hardwoods, oak-hickory	−5 to −10	150–160	good
Lordville	Sandstone, red shale and siltstone	Oquaga-Lackawanna silt loam and stony silt loam	Oak-chestnut	−15 to −20	130–160	poor

* According to Fisher *et al.* (1962).
§ According to Cline (1955) for the Ripley and Lordville areas and to Pearson *et al.* (1942) for the Seneca Army Depot.
† According to Smith (1954).
‡ According to Cornman (1941).

Lordville Area

The Lordville area of 15,124 acres is located in the southeastern part of the State along the Pennsylvania border and comprises a long-established winter deer yard. Deer that wintered there scattered out at other seasons over an area of some 320 square miles (Whalen, 1964). The terrain is mountainous, and the food supply was limited and of poor quality. For many years there had been chronic starvation, and winter mortality had reached 50 deer per square mile (Hesselton and Jones, 1963). Deer from this area were among the poorest in the State.

METHODS

The special hunts which provided data for this study were held after the regular deer season. Hunters were entitled to take one deer of either sex and were required to present their deer uneviscerated at a Conservation Department checking station for inspection before leaving the area. At this time the left eyeball of each was removed, its age was determined according to Severinghaus (1949), and its body weight was recorded. In most instances the time between death and removal of the eyeball was less than 1½ hours.

Eyes were fixed in 10 per cent formalin for approximately 7 months. Then the lenses were carefully removed as described by Friend (1967a), ovendried at 120° C. for 7 days, air cooled for 15 minutes and weighed to the nearest 0.1 milligram on a Mettler model B direct-reading balance. Lenses were dried in shell vials but were removed from the vials for cooling and weighing as these vials were found to lose weight during the drying process and regain that weight during cooling. By contrast the lens was not found to be hygroscopic (Friend, 1967a).

Upon removal from the drying oven each lens was inspected and graded. Based on appearance, they were placed into one of five categories (intact, portions of capsule missing, pitted, knicked or frozen). The imperfections noted resulted from several causes including: a cut in the sclera (muscle covering) of the eye at the time of removal that allowed the formalin to penetrate directly and cause pitting and sloughing of the outside layers of cells during the long fixation period; freezing in the fixative due to inadequate precautions against the low temperatures encountered; physical damage caused in removing the lens; and occasional splitting of lenses in the oven.

All lenses were redried and weighed 2 months after the initial weighing as a check on the accuracy of the measurements. Differences were insignificant, and in almost every instance the second

weighing produced the lower value (average difference of —1.05 milligrams with a range of +0.1 to —3.1 milligrams for a sample of 125 lenses). Values determined from the second weighing were used in the analyses for consistency.

No statistical differences could be demonstrated between the intact lenses and those in any of the other categories. Nevertheless, only intact lenses were used in subsequent analyses. This reduced the size of samples by 30 to 40 per cent in many instances, but it is felt that the loss in specimens was more than compensated for by the elimination of unmeasurable but real variations in lens weight resulting from physical damage. Sufficient specimens for analysis were available only for fawns (of both sexes) and adult females.

FINDINGS

The data for each area are summarized in Table 2. Statistical evaluation of lens weights for the Ripley Area and the Seneca Army Depot failed to show any significant differences. The data for these two areas were then combined and tested against those for the Lordville area (Table 3). Mean weights for the Lordville sample were significantly lower than those for the combined Ripley-Seneca sample for all sex and age groups represented. Similar results were obtained when the Lordville sample was tested against each of the other samples independently. The standard error of the mean difference (Paterson, 1939) was employed to determine significance.

The degree to which lens weights for different age groups overlapped was also calculated. There was no overlap between the weights for fawns and those for older deer in either the sample from Lordville or the combined sample from the other two areas. Among the older deer, however, approximately 64 per cent of the weights for 2½ year olds, 64 per cent of those for 3½ year olds and 40 per cent of those for 4½ year olds fell within the range of the weights for yearlings in the data for the Lordville area. By contrast, these proportions were 53, 17 and 13 per cent, respectively, for the other areas.

DISCUSSION AND CONCLUSIONS

The crystalline lens of the eye is unique in that its growth does not cease with that of the rest of the body but is continuous throughout the whole period of life. This observation by Smith (1883) is the basic concept upon which use of the eye lens as a criterion of age, as applied to wildlife, is based. Another important concept is that expressed by Jackson (1913) who stated that the growth of the eye may be somewhat independent of influences affecting the

TABLE 2. DRY WEIGHTS OF FORMALIN-FIXED EYE LENSES FROM DEER TAKEN ON THE THREE AREAS

Area	Number	Weight in milligrams	
		Mean	Range
Male fawns			
Ripley	36	321.8	296.2–363.4
Seneca Army Depot	18	326.3	285.7–381.3
Lordville	52	292.1	252.2–396.8
Female fawns			
Ripley	28	316.1	286.7–352.8
Seneca Army Depot	21	315.7	265.5–348.0
Lordville	34	291.3	262.6–312.3
Yearling females			
Ripley	21	446.7	388.8–564.3
Seneca Army Depot	21	445.9	376.0–513.8
Lordville	10	424.2	396.1–451.9
2½-year-old females			
Ripley	26	512.8	463.0–555.5
Seneca Army Depot	8	512.6	464.6–552.2
Lordville	23	486.9	401.7–542.3
3½-year-old females			
Ripley	19	541.7	435.9–600.2
Seneca Army Depot	4	547.5	495.3–586.3
Lordville	14	503.4	458.0–583.4
4½-year-old females			
Ripley	10	559.7	491.0–635.5
Seneca Army Depot	5	568.4	546.5–592.1
Lordville	10	518.8	421.3–586.2

growth of the body as a whole. Early investigations by Jackson (1915) and Stewart (1918) and more recently those by Friend (1967b) illustrate that growth in rats, as expressed by body weight, can be completely suppressed by underfeeding for considerable periods with no accompanying retardation in lens growth. These findings appear to contradict those of the present study. There is, however, an important difference in the conditions involved.

Table 3. Comparison of Mean Differences in Dry Weight of Eye Lenses for Deer from Good Quality Range and Poor Quality Range.

Area	Number	Mean weight (milligrams)	Difference between means
Male fawns			
Ripley and Seneca Depot	54	323.9±17.6	31.2**
Lordville	52	292.1±24.5	
Female fawns			
Ripley and Seneca Depot	49	316.0±16.1	24.7**
Lordville	34	291.3±15.6	
Yearling females			
Ripley and Seneca Depot	42	446.3±37.2	22.1*
Lordville	10	424.2±20.3	
2½ year-old females			
Ripley and Seneca Depot	34	512.7±27.8	25.8**
Lordville	23	486.9±38.9	
3½ year-old females			
Ripley and Seneca Depot	23	542.7±37.8	39.3**
Lordville	14	503.4±38.6	
4½ year-old females			
Ripley and Seneca Depot	15	562.6±37.5	43.8*
Lordville	10	518.8±45.4	

* Significant at 5 per cent level.
** Significant at 1 per cent level.

The laboratory studies dealt only with postnatal growth after weaning, and the animals used were the progeny of well nourished mothers. In contrast, the present data for deer represent specimens from areas where the parent does were subject to substantial differences in nutrition, especially during pregnancy. On the Ripley area and the Seneca Army Depot, winter food conditions were good, whereas on the Lordville area they were poor.

Starvation has been chronic in the Lordville deer yard for many years. Deer surviving the winter leave the yard in poor physical

condition, especially does which are carrying fawns. It is unlikely that the physical condition of these does improves appreciably until after the fawns have been weaned. Thus most fawns produced on that area are from mothers that were in poor physical condition at the time of parturition and remained in poor condition throughout much of the nursing period. It seems probable that the differences in lens weight recorded were the result of differences in nutrition at some stage prior to weaning.

Hesselton in a companion study (in manuscript) used various indices of nutrition (body weight, antler growth, reproductive rates and percentage of kidney fat) to show that deer from the Lordville area were in significantly poorer condition than those from either the Ripley area or Seneca Army Depot. No such differences were found between the latter, however, and they were considered comparable.

One might conjecture that, on poor range, breeding dates might be retarded. If this were true, a disproportionate number of late fawns would be produced on the Lordville area. Data for such younger-than-average deer would skew the mean lens weight downward and result in the apparent differential between this and the other two areas being wider than that actually existing. But there is no evidence of retarded breeding under such conditions in New York.

It is known, however, that little, if any, breeding takes place among fawns on poor quality range. On the other hand, a high proportion of the fawns in well nourished populations do breed. But they breed late, and their progeny are the youngest animals present the following winter. Therefore, a lens weight distribution for fawns that was skewed downward because of the presence of such specimens would be more typical of a population on good range than of one on poor range. In the present instance, the effect would be to narrow the differential between the data for the Lordville area and those for the other two areas.

Thus there was no age disparity among the fawns that might account for the differences in lens weight. Also, although the average fawning date for all deer may have been slightly earlier on the Lordville area, average ages for the various age groups on the three areas were closely similar (Severinghaus, unpub.). Therefore, the data are considered to have been comparable.

The results of this study indicate that nutritional deficiencies that adversely affect the physical condition of wild deer during the winter, spring and summer also retard eye-lens growth in fawns during their fetal period or prior to weaning. Presumably the effect

of such deficiencies is heightened where the parent females return to poor winter range year after year.

Two implications arise from this finding. First, lens weight may be useful in developing indices to game range conditions in relation to deer population density when age can be accurately determined by criteria other than lens size. Second, the nutritional status of the animals must be taken into account in any attempt to develop an index to age based on growth and size of the eye lens. Further study will be necessary before lens-weight data can be applied in this way.

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ESTIMATES OF BIOMASS AND MORTALITY RATES IN A WALLEYE POPULATION[1]

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ABSTRACT

The population of walleyes in Oneida Lake was estimated from fish marked on the spawning grounds and recaptured during the summer and fall following their dispersal. Numbers of age IV and older walleyes in April ranged from 265,700 to 1,035,000, and the estimated standing crop of age III and older fish varied from 16.5 to 27.3 pounds per acre between 1957 and 1963. Annual survival was determined from recoveries of fish marked in successive years on the spawning grounds, and estimates of the sport fishing catch during three seasons provided data needed to separate fishing and natural mortality. The highest annual mortality rate occurred in 1959 when anglers harvested about half the spring population. In other years rates of total mortality were 0.11 to 0.40. Loss of adult fish from natural causes was about 5 per cent per year.

Oneida Lake, with a surface area of 51,000 acres, is the largest lake within New York State. Annual sport fishing effort averaged about one million man hours during the years 1957 to 1959, and was mostly for walleyes (Grosslein, 1961). Extreme fluctuations in the catch during the early 1950's caused growing concern for the future of the fishery, and a study of population dynamics was undertaken in 1956. The growth rate of walleyes and the distribution and movements of the spawning populations have been described in earlier publications (Forney 1963, 1965). This paper summarizes changes in population size and analyzes their relation to recruitment and fishing mortality between 1957 and 1963.

Population size and mortality rates were estimated by marking walleyes on the spawning grounds and recapturing marked fish during the summer and fall following their dispersal. This procedure has been used successfully in smaller lakes to estimate vital statistics of walleye populations (Olson, 1958; Whitney, 1958; Rose, 1949). Estimates of the sport fishing catch during three seasons provided data needed to separate fishing mortality and natural mortality.

GENERAL PROCEDURE

Walleyes were captured for marking during the spawning migration in April. Most of the fish marked were taken in trap nets set at

[1] A contribution of Federal Aid in Fish and Wildlife Restoration Project F-17-R. The cooperation of personnel of the Oneida Fish Hatchery made possible the marking of large numbers of walleyes, and C. B. Taylor provided valuable field assistance throughout the study. Helpful comments on the manuscript were made by A. W. Eipper and C. A. Carlson.

the mouth of Scriba Creek to intercept walleyes entering this stream which is one of the two major spawning tributaries. The fish caught were fin clipped after spawn-taking operations and then released in the creek ¼ mile from the lake. Additional walleyes taken during the spawning run in trap nets at Fish Creek and by electrofishing at Dutchman's Island were fin clipped and released where captured. Those taken at Fish Creek and Dutchman's Island constituted 15 to 20 per cent of the total number marked each year.

A distinctive fin clip was used to mark fish each year. The right ventral fin was clipped in 1957, the left ventral in 1958, and both ventrals in 1959. In these years ventral fins were clipped at the base, and there was virtually no fin regeneration. In subsequent years only the distal one-half to two-thirds of either a ventral or pectoral fin was removed as a mark. Although partially clipped fins often regenerated to nearly normal length in one or two growing seasons, the line of excision could be easily recognized by swelling and distortion of the fin rays as described by Eipper and Forney (1965).

Age composition of the spawning run was determined from scales collected from about 1,000 males and an equal number of females at the time of marking. Prior to 1962 most males matured at age IV and females at age V, but in 1962 and 1963 nearly all males matured at age III and females at age IV. Consequently the youngest age group included in the population estimate was age IV from 1957 to 1961 and age III in 1962 and 1963.

All recapture data for estimating population size and annual mortality rate were obtained by netting and electrofishing, except in 1957 when walleyes observed during a creel census were also examined for marks. During the 1957 fishing season 1,043 walleyes caught by anglers were checked, and 46 of these were marked. In the same year, 43 of 858 walleyes taken by netting and electrofishing were marked. Since the ratios were not significantly different (chi-square = 0.35 with 1 degree of freedom), the population estimate for 1957 was based on the combined data. In other years approximately two-thirds of the recoveries were made by electrofishing at night in October and November, and the remaining fish were caught by trawling, trapnetting and gill netting in summer and fall. The shoreline was divided into eight segments of equal length, and the same number of hours of electrofishing was expended along each segment. Netting effort was distributed as uniformly as possible throughout the lake, but trawling was limited to deeper areas where the bottom was smooth, while trap nets were only effective in shallow water. Trap nets were not used to obtain recaptures after 1960, since their efficiency was low compared with that of electrofishing and trawling.

POPULATION ESTIMATES

The walleye population was estimated by age group to determine the size of year classes at approximately the time they entered the fishery and to eliminate the effect of recruitment between marking and recovery periods. The number of males and females in each age group marked and released was estimated from the age composition of samples taken during the spawning run. Scales were taken from all marked fish and from samples of unmarked fish caught during the summer and fall (Table 1) to determine the age composition of the catch. From these data the number in each year class at the time of marking in April ($\hat{N}$) was estimated from the familiar Petersen formula:

$$\hat{N} = \frac{MC}{R}$$

where M is the number marked, C the total number examined and R the number recaptured.

Table 1. Number of Walleyes Fin Clipped in April and the Catch and Number of Recaptures During the Following Summer and Fall

Year	Item	Age group							Total
		III	IV	V	VI	VII	VIII	Older	
1957	Number marked	82	193	6,347		5,898			12,520
	Catch	1,995	127	912		862			3,896
	Recaptures	0	2	44		43			89
1958	Number marked	389	12,354	369	4,183	3,156			20,451
	Catch	371	4,815	68	818	696			6,768
	Recaptures	1	76	2	27	19			125
1959	Number marked	111	1,474	19,746	147	2,590	1,618		25,686
	Catch	232	377	4,506	38	692	473		6,318
	Recaptures	1	10	126	1	15	10		163
1960	Number marked	163	494	961	8,780	140	1,532	1,039	13,109
	Catch	3,039	226	254	2,078	42	543	439	6,621
	Recaptures	4	3	5	56	1	16	11	96
1961	Number marked	279	10,291	1,691	1,417	9,229	146	2,328	25,381
	Catch	576	2,805	330	94	1,388	28	445	5,666
	Recaptures	3	67	11	3	57	1	13	155
1962	Number marked	3,274	1,583	12,891	1,519		5,048	1,418	25,733*
	Catch	2,125	372	2,170	140		793	281	5,881
	Recaptures	24	9	97	3		31	8	172
1963	Number marked	1,430	5,614	1,473	8,415	877		2,833	20,642
	Catch	1,763	1,809	208	1,508	192		895	6,455
	Recaptures	9	37	8	40	3		25	122

* Not including 44 specimens in age group II.

Between 12,520 and 25,777 walleyes were marked each year on the spawning grounds, and 3,896 to 6,768 were collected during the post-spawning period to determine the ratio of marked to unmarked fish. Adults marked in the spring constituted 2 to 3 per cent of the estimated population of age IV and older fish each year, but a smaller proportion was examined for marks because of the difficulty of sampling after the fish had dispersed from their spawning areas. Walleyes examined for marks were released after processing.

Population estimates were made by combining all age IV and older fish (Table 2) and by subdividing the catch by year class

TABLE 2. PETERSEN ESTIMATES OF AGE IV AND OLDER WALLEYES IN APRIL

Year	Number marked	Catch	Recaptures	Population	Confidence interval (95 per cent)
1957	12,438	1,901	89	265,700	216,000– 327,000
1958	20,062	6,397	124	1,035,000	868,000–1,234,000
1959	25,575	6,086	162	960,800	824,000–1,121,000
1960	12,946	3,582	92	504,000	411,000– 618,000
1961	25,102	5,090	152	840,600	717,000– 985,000
1962	22,459	3,756	148	570,000	485,000– 670,000
1963	19,212	4,692	113	797,700	664,000– 959,000

(Table 3). The sums of the estimates by year class were similar to estimates calculated from the combined data which suggests that fish of different ages were equally vulnerable to capture. Some year classes were poorly represented in the spawning run, and the numbers marked and recovered were inadequate to provide reliable estimates of year-class size (Table 1). However, estimates of these year classes are shown since they provide an indication of the variation in year-class size.

Estimates of the population of age IV and older fish were based on recoveries of 89 to 162 marked fish. Confidence intervals for samples of this size are relatively broad (Table 2), but differences between annual population estimates were large enough to be considered significant. Following is an appraisal of the accuracy of these estimates.

Studies of gear selectivity, loss of marks, movements and distribution of the population suggest that the assumptions on which the Petersen estimate is based were not seriously violated. The age composition of the catch in trapnets during the spawning run accurately reflected the age distribution of the spawning population (Forney, 1961). The age distribution of fish recaptured by electro-

Table 3. Petersen Estimates of Size of Year Classes of Walleyes at the Time of Marking in April From 1957 Through 1963

Year	Year class										Population of age IV and older fish
	1951 and earlier	1952	1953	1954	1955	1956	1957	1958	1959	1960	
1957	118,200	131,600	12,300	..	..	.	..	.	..	..	262,100
1958	115,600	126,700	12,500	782,800	..	.	.	.	..	..	1,037,600
1959	76,500	119,500	5,600	706,200	55,600	.	.	..	..	..	963,400
1960	41,500	52,000	5,900	325,800	48,800	37,200	..	.	..	..	511,200
1961	79,700		4,100	224,700	44,400	50,700	430,900	..	..	..	834,500
1962	49,800			129,100	70,900		288,400	65,400	290,000	..	603,600
1963	101,400				56,100		317,200	53,000	274,500	280,100	802,200

TABLE 4. ESTIMATE OF THE NUMBER OF AGE IV AND OLDER WALLEYES IN APRIL 1958 FROM RECOVERIES IN 1958, 1959 AND 1960

Year	Age group	Catch	Recaptures*	Estimated population in 1958
1958	IV and older	6,397	124	1,035,000
1959	V and older	5,709	109	1,050,800
1960	VI and older	3,102	50	1,244,600

* Of fish marked in 1958.

fishing and trawling was similar to that of marked fish released in the spring, which established that the sampling gear was not selective.

Since the population was estimated by year class, recruitment of catchable-sized fish into the population between marking and recovery did not have to be considered. Close agreement between estimates of the April 1958 population based on the catch in 1958, 1959 and 1960 showed that the age composition of the catch was accurately assessed from scale samples (Table 4). The similarity between the marked-unmarked ratio in the catch also implied that fin-clipped and unmarked fish experienced about the same mortality between successive sampling periods. Error from failure to recognize marked fish was considered negligible, since fins clipped at the base seldom regenerated, and partial fin clips could be easily recognized by trained observers (Eipper and Forney, 1965).

The most serious potential source of error in estimating the population was the failure of marked and unmarked fish to become randomly mixed. Angling recoveries during the summer and fall of fish tagged on the spawning grounds indicated that populations dispersed widely after spawning. However, analysis of the relative spatial distribution of tag recoveries from three spawning populations demonstrated that mixing was only partial (Forney, 1963). Since 80 to 85 per cent of the fish marked to estimate the population were caught at Scriba Creek, the ratio of marked to unmarked fish was not uniform throughout the lake. A reasonably uniform distribution of recapture effort was achieved by conducting electrofishing along the entire shoreline and spacing netting sites throughout the lake basin. Population estimates from 1958 through 1960 based on the catch in different gear were similar (Table 5) and supported the assumption that the scattered distribution of recapture effort compensated for the non-random distribution of marked fish.

Independent estimates of the population of walleyes in the fall of 1958, 1959 and 1960 were made to further verify the accuracy

Table 5. Number of Age IV and Older Walleyes in April As Estimated From the Catch in Several Types of Gear

Gear	Catch	Recaptures	Population
1958			
Electrofishing	3,961	82	967,000
Trawl	762	15	1,017,000
Gill net & trapnet	1,674	27	1,243,000
1959			
Electrofishing	3,322	104	816,900
Trawl	1,277	27	1,210,000
Gillnet & trapnet	1,487	31	1,227,000
1960			
Electrofishing	1,840	49	486,000
Trawl	897	17	683,000
Gillnet & trapnet	845	26	421,000

of spring estimates. Walleyes collected by electrofishing and trapnetting in October and November of 1958, 1959 and 1960 were marked by removing part of the dorsal or anal fin. Samples of the catch were killed to determine the sex ratio, and scales were collected to estimate the age of the fish marked. The fall population of age III+ and older walleyes was estimated from recoveries of marked fish in spawning runs at Scriba Creek, Fish Creek and Dutchman's Island during the following spring. Chi-square tests of homogeneity indicated that the ratios of marked to unmarked fish in the samples inspected at the three locations were not significantly different, so the catches from all the areas were pooled to estimate the fall population (Table 6).

Fall population estimates are not directly comparable to spring estimates for age IV and older fish, since angling occurred between October and April. From a creel census of ice fishermen (Grosslein, 1961) the total catch of age III+ and older fish was estimated at 57,000 in the winter of 1958–59 and 50,000 in the winter of 1959–60. No creal census was conducted in the winter of 1960–61, but the angling catch for that winter was estimated at about 15,000 based on the rate of exploitation from angling returns and the estimated fall population. As will be shown later, the annual instantaneous natural mortality rate was about 0.06, and the mortality rate for the 6-month period from November to April would have been 0.03

TABLE 6. PETERSEN ESTIMATES OF THE POPULATION OF AGE III+ AND OLDER WALLEYES IN OCTOBER

Year	Sex	Number marked	Catch		Estimated population
			Total	Recaptures	
1958	Male	2,003	12,644	58	436,700
	Female	3,412	8,776	62	482,900
	Total				919,600
1959	Male	1,435	7,157	36	285,300
	Female	2,152	3,755	39	207,100
	Total				492,400
1960	Male	1,937	15,710	77	395,200
	Female	2,833	7,054	50	399,700
	Total				794,900

TABLE 7. ESTIMATED AND EXPECTED POPULATIONS OF AGE IV AND OLDER WALLEYES IN APRIL

Year	Estimated population*	Expected population§
1959	960,800	840,000
1960	504,000	430,000
1961	840,600	760,000

* Calculated from fish marked in spawning run.
§ Calculated by subtracting overwinter loss from Petersen estimate for age III+ and older fish in preceding October.

if the natural mortality rate was constant. Using rates of exploitation calculated from the winter catch and fall population estimates, and assuming fishing was an exponential function, estimates of total mortality were obtained by trial and error iteration.

The expected spring populations were obtained by subtracting overwinter mortality from the Petersen estimates of the fall population of age III+ and older walleyes. Expected spring populations, calculated in this manner, were slightly lower than estimates of the April population of age IV and older walleyes determined from fish marked in the spawning run (Table 7). However, agreement was sufficiently close to confirm that the procedure for estimating the spring population was valid.

SURVIVAL

Survival of walleyes from April to the following April was

estimated from recoveries of fish marked in successive years on the spawning grounds. If M_1 represents the number of fish marked at the beginning of the fishing season in the first year and M_2 the number marked a year later, the survival (S_1) between marking periods can be estimated from the relation

$$S_1 = \frac{(R_{12} + R_{13} \ldots R_{1n})\ M_2}{(R_{22} + R_{23} \ldots R_{2n})\ M_1}$$

where $R_{12}/R_{22} \ldots R_{1n}/R_{2n}$ are the ratios of first-year to second-year marks observed in recaptures during the second and subsequent years. Estimates of the ratio R_{1n}/R_{2n} were available from recaptures in the spawning run at Scriba Creek and from recoveries in nets and by electrofishing during summer and fall.

Beginning in 1959, samples of the fish netted in April at Scriba Creek were examined for marks. Recaptures of marked fish were grouped by sex and then classified according to year of marking and year of recovery. All recaptures were aged from scales, and the number of marked fish in each year class was estimated from the age composition of samples taken during the spawning run (Table 8). Using these data and the foregoing formula, survival was estimated for males (females were not included for reasons given later) which were age IV or older in the first year of marking and age V or older in the second year (Table 9). By comparing recoveries from the same year classes, possible differences in vulnerability of year

Table 8. Number of Male Walleyes in Certain Year Classes Marked at Scriba Creek During the Spawning Run and Recaptures Observed in Subsequent Spawning Runs

Year class	Year marked	Number marked	Number recovered						
			1959	1960	1961	1962	1963	1964	Total
1953 and earlier	1957	9,912	303	177	185	113	50	28	856
	1958	4,416	181	89	102	57	34	11	474
1954 and earlier	1958	15,499	..	271	294	198	102	34	899
	1959	11,719	..	232	247	167	83	34	763
1955 and earlier	1959	12,499	..	..	252	171	86	37	546
	1960	7,732	..	..	337	234	124	46	741
1956 and earlier	1960	8,136	..	..	..	252	141	45	438
	1961	6,666	..	..	..	361	173	64	598
1957 and earlier	1961	14,750	..	..	..	..	406	218	624
	1962	9,651	..	..	..	..	348	201	549
1958 and earlier	1962	10,937	..	..	..	..	..	216	216
	1963	6,028	..	..	..	..	..	147	147

Table 9. Survival Rate From April to April As Estimated From Recaptures of Marked Walleyes in the Spawning Run and From Recaptures During the Post-spawning Period

Period	Survival rate estimated from	
	Spawning run*	Post-spawning Period
1957–58	0.80	1.12
1958–59	0.89	0.86
1959–60	0.46	0.42
1960–61	0.60	0.66
1961–62	0.74	0.62
1962–63	0.81	0.73

* Males only.

classes to recapture and any differences in mortality rate associated with age were minimized.

Estimates of survival were based on the numbers of walleyes marked and recovered at Scriba Creek, although smaller numbers of those spawning at Dutchman's Island and Fish Creek were marked each year with the same fin clip used at Scriba Creek. This seems to have been justified, since fish which were tagged on each of the three spawning grounds tended to return to the same spawning area in successive years. In samples from the 1958 to 1961 spawning runs, 274 tagged fish which had been at large for one or more years were recovered. Of these, 245 were recovered at the site of original release, while the remaining 29 had strayed to different spawning areas.

In samples of walleyes examined at Scriba Creek, the proportion of marked to unmarked females in the same year classes varied from year to year (Table 10). Apparently the groups of females marked during successive spawning runs were not equally vulnerable to recapture in subsequent spawning runs.

Trap nets set at the mouth of Scriba Creek may sample populations spawning in the lake in addition to those entering the stream. Differences in stream and lake temperatures, lake currents or other factors may determine the composition of the sample marked and the probability of females being recaptured in the following spawning run. Failure of some females to spawn each year after reaching sexual maturity may contribute to changes in the proportion of marked fish recovered (Forney, 1965). Males apparently constituted a much more homogeneous group than females since the ratio of marked to unmarked males was reasonably similar in successive spawning runs (Table 11). This may have been attributable to the tendency of males to remain on the spawning grounds throughout the run or to greater intermingling of males among spawning areas near Scriba Creek.

Table 10. Number of Female Walleyes Marked in the 1957 to 1961 Spawning Runs at Scriba Creek Which Were Observed in Samples Collected in Subsequent Spawning Runs*

Year marked	Item	Year of collection				
		1959	1960	1961	1962	1963
1957	Number in sample	1,363	1,152	1,250	806	214
	Number marked	166	44	35	24	17
	Proportion	0.1218	0.0382	0.0280	0.0298	0.0794
1958	Number in sample	6,423	3,236	4,994	3,128	1,322
	Number marked	237	131	95	67	37
	Proportion	0.0369	0.0405	0.0190	0.0214	0.0280
1959	Number in sample	..	3,431	5,341	3,435	1,492
	Number marked	..	190	258	164	71
	Proportion	..	0.0554	0.0483	0.0477	0.0476
1960	Number in sample	..	..	5,618	3,826	1,718
	Number marked	..	..	216	142	70
	Proportion	..	..	0.0384	0.0371	0.0407
1961	Number in sample	..	..	..	7,837	5,500
	Number marked	..	..	..	456	243
	Proportion	..	..	..	0.0582	0.0442

* Samples included only specimens which had been age IV or older at the time of marking.

Table 11. Number of Male Walleyes Marked in the 1957 to 1961 Spawning Runs at Scriba Creek Which Were Observed in Samples Collected in Subsequent Spawning Runs*

Year marked	Item	Year of collection				
		1959	1960	1961	1962	1963
1957	Number in sample	2,067	1,193	1,117	661	305
	Number marked	303	177	185	113	50
	Proportion	0.1466	0.1484	0.1656	0.1709	0.1639
1958	Number in sample	9,850	5,355	4,890	2,888	1,637
	Number marked	705	271	294	198	102
	Proportion	0.0716	0.0506	0.0601	0.0686	0.0623
1959	Number in sample	..	5,807	5.711	2,995	1,766
	Number marked	..	247	252	171	86
	Proportion	..	0.0425	0.0441	0.0571	0.0487
1960	Number in sample	..	..	6,706	3,248	1,988
	Number marked	..	..	361	252	141
	Proportion	..	..	0.0538	0.0776	9.0709
1961	Number in sample	..	..	..	8,422	4,969
	Number marked	..	..	..	844	406
	Proportion	..	..	..	0.1002	0.0817

* Samples included only specimens which had been age IV or older at the time of marking.

TABLE 12. RECAPTURES OF WALLEYES MARKED DURING THE SPAWNING RUN AND COLLECTED IN EXPERIMENTAL GEAR, CLASSIFIED ACCORDING TO YEAR MARKED AND YEAR RECOVERED

Year marked	Number marked	Number recovered							
		1957	1958	1959	1960	1961	1962	1963	Total
1957	12,520	89	88	59	47	24	16	8	331
1958	20,451	..	125	111	55	27	24	10	352
1959	25,686	..	..	163	80	37	29	23	332
1960	13,109	..	..	..	96	58	28	25	207
1961	25,381	..	..	..	..	155	95	77	327
1962	25,777	..	..	..	..	..	172	111	283
1963	20,642	..	..	..	..	..	..	122	122

A second, and partly independent, estimate of annual survival can be made from walleyes marked on the spawning grounds and recaptured by electrofishing and netting in summer and fall. All fish collected were examined for marks, and recaptures were classified according to year of marking and year of recovery (Table 12). Survival was estimated from the total number marked each spring and the total number recovered from 1958 through 1963. Estimates of survival by this method and from recaptures in the spawning run was similar except in 1957.

Walleyes marked during the 1957 spawning run appeared more vulnerable to recapture during the post-spawning period than fish marked in subsequent years. Comparison of recaptures of fish marked in 1957 with recoveries of walleyes marked in later years did not reveal any significant differences in their distribution which could account for the high proportion recovered, but the 1957 spawning run was unique. Water temperatures in Scriba Creek during 1957 were lower than lake temperatures, and very few walleyes entered the stream. Although substantial numbers were taken in nets at the mouth of the creek, the catch may not have been representative of the Scriba Creek spawning population. But, fish marked in 1957 did enter subsequent spawning runs in about the expected numbers.

The generally close agreement between estimates of survival from recaptures at different times of the year supports the conclusion that the methods employed were accurate. Further evidence that the survival estimates were approximately correct can be inferred from estimates of exploitation described under the following topic.

RATES OF EXPLOITATION AND NATURAL MORTALITY

The annual rate of exploitation can be expressed as that fraction of the population present at the beginning of the year which

is harvested by anglers during the following open season. The angling catch during the open season (May 1 to March 1) was estimated from a creel census conducted from May 1957 through February 1960 (Grosslein, 1961). The estimated total catch was 40,000 in 1957–58, 112,000 in 1958–59 and 463,000 in 1959–60. From scales taken during the creel census it was estimated that, of these, 32,000, 106,000 and 449,000, respectively, were age IV or older. Rates of exploitation obtained by dividing the latter figures by the estimated numbers present in April were 0.14, 0.10 and 0.47, respectively.

The number of deaths from natural causes can be estimated by substracting, from the April population estimate of age IV and older fish, the number of age V and older fish remaining one year later and the angling catch during the intervening fishing season (Table 13). The difference between the estimated population of age IV and older fish in April 1957 and that of age V and older fish in April 1958 was less than the estimated catch during the 1957–58 fishing season. Possibly the population estimates for 1957 and 1958 were not comparable, since estimates of survival indicated that the fish marked in 1957 were more vulnerable to capture than those marked in subsequent years. However, it does appear that natural mortality was low between 1957 and 1958. Reasonable estimates of natural deaths can be obtained for other years. Dividing the natural losses by the spring population, the expectation of death from natural causes (v) as defined by Ricker (1958) was 0.02 for 1958–59 and 0.4 for 1959–60.

Estimates of v can also be obtained by subtracting the rate of exploitation from the total annual mortality. Estimates of annual survival from recaptures in the spawning run are shown in Table 9, and the corresponding total mortality rates were 0.20 for 1957–58, 0.11 for 1958–59 and 0.54 for 1959–60. Subtracting the rates of exploitation, the expectation of death from natural causes (v) was 0.06, 0.01 and 0.07, respectively, for the three 12-month periods from April 1957 to April 1960. If survival estimates are relatively more reliable than population estimates, subtracting the rate of exploitation would provide a more precise estimate of v, particularly for 1957 and 1958 when the catch was obviously a small fraction of the spring population.

If mortality components are expressed as instantaneous rates (Ricker, 1958), the calculated coefficient of natural mortality (q) would be 0.07 for 1957–58, 0.01 for 1958–59 and 0.10 for 1959–60. Assuming natural mortality was constant, and year to year fluctuations in q resulted from non-systematic errors in estimates of population parameters, the mean q of 0.06 is the best estimate of natural mortality. This value is close to the estimated q of 0.0491

TABLE 13. LOSS OF WALLEYES BETWEEN ANNUAL POPULATION ESTIMATES AND DISTRIBUTION OF LOSS BETWEEN ANGLING CATCH AND NATURAL MORTALITY

Year class	Population estimate		Loss	Distribution of loss	
	April 1957	April 1958		Angling catch	Natural mortality
1953 and earlier....	265,700	254,000	11,700	32,000*	..
1954 and earlier..	1,035,000	905,200	129,800	106,000	23,800
1955 and earlier....	960,800	469,500	491,300	449,000	42,300

* Greater than estimated loss (see text).

which Olson (1958) reported for age IV and older walleyes in Many Point Lake, Minnesota.

STANDING CROP

The standing crop in April was estimated by multiplying the number in a year class by their mean weight and summing the contributions of the various year classes which were age III or older. The mean length for each year class in April was calculated from scales taken in various gear during the summer, and it was converted to mean weight for an age group by using a "between ages" length-weight regression (Ricker, 1958, p. 191) based on weight measurements taken during the summer for the years 1957 to 1962. Standing crops on July 1 and November 1 were estimated to provide an indication of fluctuations between annual inventories. The numbers of walleyes surviving from April 1 to July 1 and to November 1 were estimated by subdividing the total annual mortality determined from successive population estimates. A constant coefficient of natural mortality was assumed, and fishing mortality was apportioned between periods in the same ratio as anglers' catches of tagged walleyes during the year. Growth was considered limited to the period from July 1 to November 1. This is not precisely true since annulus formation usually occurs in late June, but less than 15 per cent of the annual growth increment occurred prior to July in any year.

In 1962 and 1963 large numbers of age III walleyes were marked during the spawning run, and a direct estimate of the population of 3-year-old fish was possible. In earlier years few fish matured at age III, and it was necessary to estimate the number from the population of age IV fish the following spring. Estimates were made by assuming that fish between ages III and IV experienced one-half the fishing mortality, and the same natural mortality,

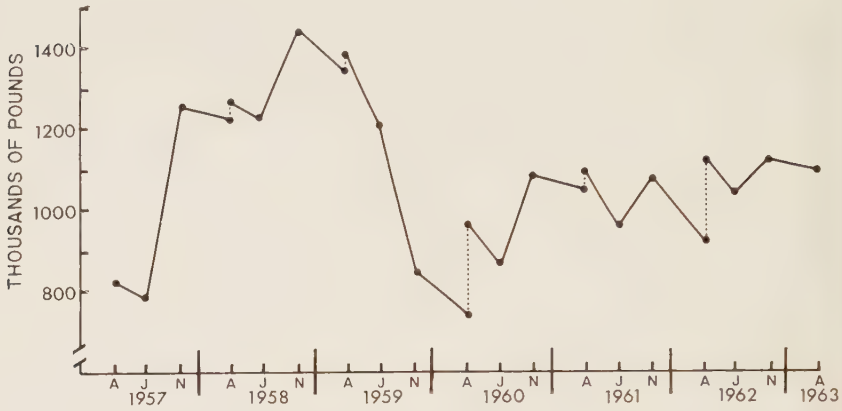

Figure 1. Standing crop of age III and older walleyes on April 1, July 1 and November 1 from 1957 to 1963.

as older fish. Tag returns indicated age III walleyes were substantially less vulnerable to angling than older fish, but the assumption that the natural mortality rate was the same as for older fish cannot be verified.

The estimated biomass of age III and older walleyes in April ranged from 825,000 pounds in 1957 to 1,365,000 pounds in 1959 or about 16.5 to 27.3 pounds per acre (Figure 1). In each year except 1959 the population showed a net gain in biomass between July and November. In 1959, slow growth and a high rate of exploitation caused a precipitous decline in the standing crop.

DISCUSSION

During the early 1950's fishermen reported extreme fluctuations in angling success for walleyes. Estimates of the catch rate are not available for this period, but tag returns did indicate unusual variation in rates of exploitation. Instability of the fishery was still evident during the seasons of 1957-58 to 1959-60 when the creel census was conducted. Catch per boat hour (from May 1 to October 31) increased slightly from 0.10 in 1957 to 0.16 in 1958 and rose to 0.71 in 1959, although population estimates indicated a small decline in the number of walleyes between April of 1958 and 1959.

The difference in catch rate between 1958 and 1959 was largely attributable to increased vulnerability to angling since in both years most anglers fished for walleyes. Vulnerability was apparently determined by the abundance of forage fish. This is suggested by the inverse relationship between the rates of exploitation

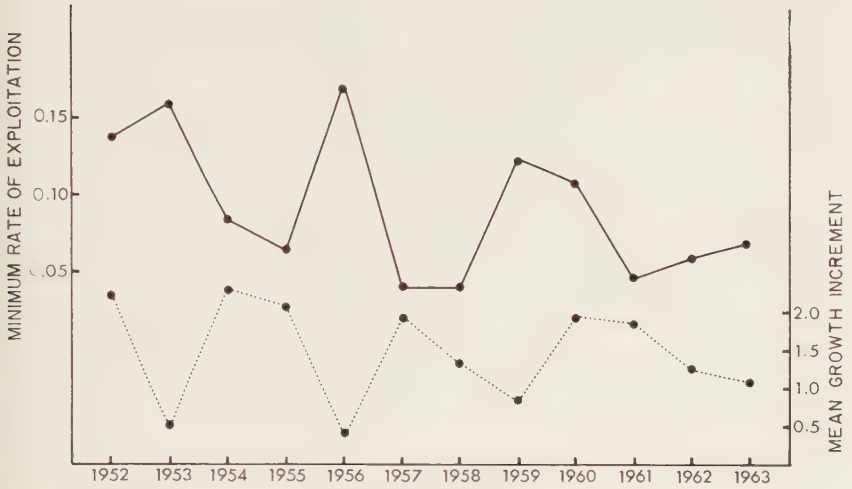

Figure 2. Mean growth increments for age IV to VI walleyes and minimum rates of exploitation based on tag returns from 1952 to 1963.

from voluntary tag returns and the annual growth increments of walleyes between 1952 and 1962 (Figure 2).

The walleye is not generally considered a species which is highly vulnerable to angling, but in 1959 an angling effort of about 25 man hours per acre harvested nearly half the spring standing crop. Even higher rates of exploitation may have occurred in earlier years judging from voluntary tag returns. The minimum rate of exploitation in 1959 was 0.14, compared with 0.17 in 1956 and 0.15 in 1953. The catch of 463,000 in 1959 resulted in a low population density in the spring of 1960, and the population was at an even lower level in April 1957 following a successful fishing season in 1956.

The sport fishery during the 1950's may have perpetuated fluctuations in the catch by periodically removing a high proportion of the population. A large catch would reduce competition for forage and the vulnerability of the remaining fish to angling, until the biomass of the population again increased. Rapid growth of walleyes in 1954, 1957 and 1960, following years when exploitation was high (Figure 2), suggests that the high catch did reduce food competition. However, it is possible that annual differences in the spawning success of yellow perch, the principle forage fish in Oneida Lake, may have been equally important in determining the vulnerability of walleyes to angling during the fishing season. Moyle (1949) observed that fishing success in Minnesota lakes was affected by the density of forage fish, and he suggested that the

harvest of walleyes would be highest when the predator population was at or above the carrying capacity and the growth rate was slow.

Implicit in the concept of managing a fishery for maximum sustained yield are the assumptions that the catch can be regulated and that the biomass of the exploited population can be held at a level consistent with optimum productivity. The annual catch of walleyes in Oneida Lake is governed by the vulnerability of the fish to angling, and fishing pressure and fishing regulations are of secondary importance in determining the catch. Since the fishery is only capable of harvesting walleyes when food competition is intense, liberal fishing regulations would probably produce the highest yields. If recruitment of young was adequate, the population would tend to return to its original level of abundance following years of high angling exploitation, and this level would probably be fixed by the amount of forage available.

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EFFECTS OF SITE EXPOSURE AND DEER BROWSING UPON THE QUANTITY AND PROTEIN-QUALITY OF WITCHHOBBLE BROWSE[1]

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ABSTRACT

The nature of witchhobble browse was studied in a spruce flat–mixed wood habitat on the Huntington Wildlife Forest in the Adirondack region of New York. Quantity of browse was positively correlated with site exposure, apparently because snow accumulation on sites exposed by openings in the tree canopy prohibited excessive browsing by deer. Crude-protein concentration in both bud- and stem-portions of browse was negatively correlated with the quantity of browse per milacre. It is suggested that reduction in browse quantity caused by excessive browsing is, to a small degree, compensated by an increase in nutrient-concentration within the browse.

Witchhobble (*Viburnum alnifolium*) is an important winter food of white-tailed deer (*Odocoileus virginianus*) in the Adirondack region of New York because it is common in their winter habitat (Barick, 1950), readily eaten, and able to survive despite severe annual browsing. In the Deer Creek drainage, a winter concentration area for deer on the Huntington Wildlife Forest near Newcomb, the physiognomy of witchhobble plants varies with site exposure. On sites exposed by small openings in the forest canopy, stand density tends to be greater and plants taller (often over 6 feet) than on shaded sites where plants are heavily browsed and rarely more than 18 inches tall. This study was designed to measure relationships among exposure, quantity of witchhobble browse, and concentration of crude protein in the browse.

METHODS

Ten clumps of witchhobble, representing a wide range of exposure conditions on level ground within a spruce flat–mixed wood cover type (Braun, 1950) in the Deer Creek area, were selected during the summer of 1962. Small enclosures were erected to protect plants within eight of the 10 clumps from deer.

An index to site exposure was obtained at each clump, using Livingston atmometers (Livingston, 1935), during the summer of 1963. Full sunlight was measured as the difference between the amount

[1] This research was conducted during preparation of a thesis to be submitted to the State University of New York College of Forestry. The aid and suggestions of Dr. E. F. Patric of the Huntington Forest was acknowledged. Thanks are also due to Dr. G. C. Sanderson, Mrs. H. C. Schultz and G. G. Montgomery, all of the Illinois Natural History Survey, for critical review of the manuscript.

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of water evaporated from a black atmometer and the amount evaporated from a white atmometer at a nearby dry lake bed. Sunlight was measured in the same manner at each clump during periods of from 10 to 26 days. Site exposure was expressed as a percentage of full sunlight, being the ratio of the difference in evaporation (between black and white atmometers) at a witchhobble clump to the difference at the dry lake bed for the same period of time.

Between June 25 and July 5 in 1963, browse quantity was measured by counting the number of terminal buds (all buds occurring at the ends of twigs) occurring on all stems originating at the ground surface within milacre plots established in each of the 10 clumps of witchhobble.

The tips of three prominent twigs with vegetative buds were collected from each of the 10 clumps on January 4, 1964. Each tip was clipped 1 inch behind the base of the terminal bud. After drying at 65° C., the bud and first inch of stem from each tip were separated and analyzed for their content of nitrogen by the Kjeldahl procedure (Wilde and Voigt, 1959). Crude-protein concentration was expressed as 6.25 times the concentration of nitrogen on an ovendry weight basis.

Crude-protein concentrations were obtained for the bud- and stem-portions of each piece of browse. A weighted average of these concentrations was used to determine the concentration of crude protein in each whole piece of browse. Protein data were averaged for each clump, and a square-root transformation was applied to these averages before analysis by regression and multiple correlation procedures.

RESULTS

Degree of site exposure, quantity of browse per milacre and average concentration of crude protein in the browse at 10 clumps of witchhobble plants are presented in Table 1. Site exposure varied from an unmeasurable amount (given a value of 1 per cent) to 39 per cent of full sunlight. Quantity of browse varied from 224 twigs with terminal buds per milacre on the most shaded site to almost three times that amount on more exposed sites. Concentration of crude protein in the browse decreased as the degree of exposure and quantity of browse increased (Figure 1). An inverse relationship between crude-protein concentration and site exposure occurred in both the bud- and stem-portions of the browse (Table 1).

There was a highly significant positive correlation ($r = +0.845$) between quantity of witchhobble browse per milacre and site

TABLE 1. RELATIONSHIPS AMONG SITE EXPOSURE, BROWSE QUANTITY AND CONCENTRATION OF CRUDE PROTEIN IN WITCHHOBBLE BROWSE FROM 10 LOCATIONS IN THE DEER CREEK AREA.

Site exposure*	Browse quantity (tips per milacre)	Crude-protein concentration (percentage of ovendry weight)		
		Whole browse	Bud portion	Stem portion
1§	224	7.44	9.10	5.69
5	281	7.12	8.19	5.77
10	281	7.40	8.88	5.75
13	313	7.77	8.85	5.96
14	248	6.75	7.71	5.48
15	272	7.83	8.79	6.25
23	399	6.96	8.08	4.92
30	630	6.48	7.31	4.91
30	658	6.42	7.37	5.15
39	497	6.79	7.96	4.91

* As percentage of full sunlight (see text).
§ No sunlight could be measured by atmometers at this very shaded location; the value of 1 per cent was assigned arbitrarily.

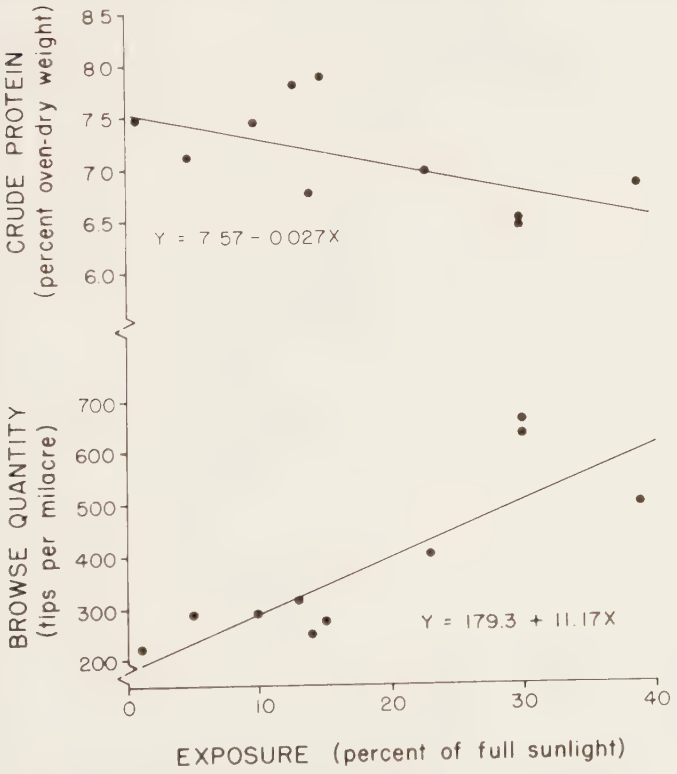

Figure 1. Relationships among site exposure, browse quantity and concentration of crude protein in witchhobble browse from 10 locations in the Deer Creek area.

exposure (Table 2). This relationship appeared to be related to snow interception by the forest canopy. During the winters of this study, snow accumulated on sites without overhead canopies to depths which prohibited travel by deer, and it was evident that plants within these small openings had, in recent years, been browsed very little compared with the severely browsed plants on shaded sites. It was, therefore, concluded that the large quantities of witchhobble browse on exposed sites had resulted primarily from a reduced intensity of deer browsing.

Concentration of crude protein in the browse was negatively correlated with both browse quantity ($r = -0.760$) and site exposure ($r = -0.645$). However, partial correlation coefficients (Table 2) indicate that the concentration of crude protein in the browse was primarily associated with the quantity of browse per milacre and only indirectly associated with site exposure, a result of the consistent association of browse quantity with site exposure. Standard partial regression coefficients (Table 2) indicate that the quantity of browse per milacre was approximately six times as important in determining crude-protein concentration in browse as was site exposure.

Concentrations of crude protein in both bud- and stem-portions of the witchhobble browse were negatively correlated with quantity of browse per milacre (Figure 2).

For purposes of illustration, witchhobble plants on six shaded sites (those receiving less than 20 per cent full sunlight) produced an average of 270 browse tips, containing an average of 7.38 per cent crude protein, per milacre. Witchhobble plants on four exposed sites (those receiving between 23 and 39 per cent full sunlight) produced an average of 546 browse tips, containing an average of 6.66 per cent crude protein, per milacre. In this comparison, browse quantity was about 50 per cent less on the shaded, intensively browsed sites than on the more exposed sites; whereas browse quality, in terms of protein concentration, was 11 per cent higher on the shaded sites than on the exposed sites.

DISCUSSION

Norman (1955) noted that factors limiting plant growth may favor concentration of nutrients (which are not limiting factors) in the plant. This principle can explain why the intensively browsed, slow-growing witchhobble plants on shaded sites produced browse having higher concentrations of crude protein than did the seldom browsed, fast-growing plants on exposed sites. However, the relative degrees to which microclimatic and edaphic factors associated with shade, and damage from severe browsing, have

TABLE 2. MULTIPLE CORRELATION ANALYSIS OF DATA FROM TABLE 1.

Item	Value
Simple correlation coefficient	
Browse quantity and site exposure	+0.845**
Protein concentration and browse quantity	−0.760*
Protein concentration and site exposure	−0.645*
Partial correlation coefficient	
Protein concentration and site exposure	−0.008
Protein concentration and browse quantity	−0.526
Multiple correlation coefficient for protein concentration, site exposure and browse quantity§	0.76*
Standard partial regression coefficient	
Protein concentration and site exposure	1.27
Protein concentration and browse quantity	7.47

* Significant at 5 per cent level.
** Significant at 1 per cent level.
§ According to formula: $Y = 3.01 - 0.0099X_1 - 0.00044X_2$ in which Y = square root of crude-protein concentration in whole browse, X_1 = degree of site exposure and X_2 = quantity of browse per milacre.

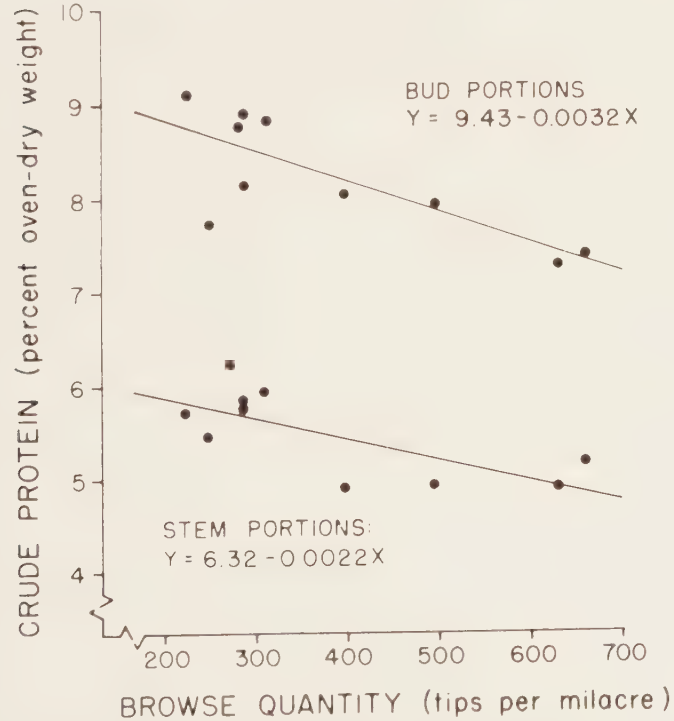

Figure 2. Crude-protein concentration in bud- and stem-portions of witchhobble browse in relation to quantity of browse per milacre.

restricted the growth of these plants are not known. It is suggested that reduction in browse quantity caused by excessive browsing is, to a small degree, compensated by an increase in nutrient-concentration within the browse.

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RELATION OF FISH GROWTH TO THE DURABILITY OF TWO DYES IN JAW-INJECTED TROUT[1]

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ABSTRACT

National Fast Blue 8GXM and hydrated chromium oxide were jaw-injected into brook trout and brown trout. Permanence of the dye marks was dependent on growth rate and independent of time. National Fast Blue 8GXM was permanent on all trout whenever gain in weight did not exceed 200 per cent. In some cases, fading and eventual extinction accompanied greater gains in weight, but in one field test clearly visible marks were retained on brook trout for which weight gains averaged 375 per cent. Hydrated chromium oxide was more variable, and extinction occurred when weight gain was between 63 and 277 per cent. However, one trout possessed a good quality mark after gaining 650 per cent in weight, and in another test well defined concealed marks were found. In the light of this evidence, hydrated chromium oxide seems the best prospect for a lifetime mark in trout. National Fast Blue 8GXM will persist for more than 2 years if growth is not excessive.

Both National Fast Blue 8GXM and hydrated chromium oxide are excellent dyes for marking trout by jaw injection. They are easily formulated, inexpensive, non-encumbering, non-toxic and non-mutilating. The author (1967) reported that durability of a dye mark depends upon two factors: the marking technique and growth of the fish between the time of marking and recapture. Good technique requires a shallow injection, and this is invariably assured by taking the simple precaution of noting whether the shadow of the needle is visible as a dark line under the skin prior to releasing the dye. However, growth of fish is highly variable and depends upon the influence of a number of individual and environmental factors. Under both laboratory and field conditions, tests of National Fast Blue 8GXM and hydrated chromium oxide showed that, at the end of identical periods of time, slow-growing fish tended to have well defined, easily visible marks while fast-growing fish tended to possess faded, more obscure marks. There were also specific differences in permanence between the two dyes over identical time periods. In laboratory studies National Fast Blue 8GXM persisted for 28 months on all brown trout marked. Under the same conditions, hydrated chromium oxide persisted on all fish for 1 year, after which the marks became extinct in a few fish. At 2 years the marks were no longer visible in 16 per cent of the marked specimens, and at 26 months only half of the marked fish possessed an

[1] A contribution of Federal Aid in Fish and Wildlife Restoration Project F-9-R. The field tests were conducted through the cooperation of the Department of Conservation at Cornell University. William Flick collected the field data for Long and Laramie Ponds.

externally visible dye mark. National Fast Blue 8GXM and hydrated chromium oxide were used at concentrations of 65 and 75 milligrams, respectively, of dye per milliliter of distilled water. These preparations are equivalent to concentrations of about 6.1 and 7.0 per cent.

A dye mark was considered extinct when it was no longer externally visible. For visible marks, histological sectioning indicated that, as growth progressed, external visibility of both dyes was diminished by a thickening of overlying skin layers and an increase in dense, fibrous connective tissue at the original site of dye disposition.

Both the laboratory experiments with brown trout and the field experiments with brook trout were continued. The present paper presents additional findings which relate to the durability of these dyes under the two test conditions and also the results of another field experiment with National Fast Blue 8GXM where the marks disappeared in a population of small, fast-growing brook trout within a year.

DURABILITY OF NATIONAL FAST BLUE 8GXM

Assuming that the fading and eventual extinction of a dye mark is directly related to the growth of the fish or, more specifically, to its growth in terms of percentage gain in weight, then it is preferable to follow the history of growth versus mark quality for individual fish rather than for a group of dyed specimens as a whole. Percentage gain in weight is determined from the following formula:

$$\text{percentage gain in weight} = \left(\frac{\text{weight at end of interval}}{\text{original weight}} - 1 \right) 100$$

For the laboratory experiments, these figures were rather crude because the gains were based on mean weights rather than individual weights. Those for the field experiments with brook trout were considerably more precise because each fish bore a serially-numbered jaw tag, and individual weights and lengths were recorded.

In all of these experiments, the quality of the dye marks was subjectively appraised as good, poor or absent. Good marks were those which were readily visible; such a mark would be immediately apparent to an observer at a distance of 6 feet from the fish. Poor marks were faintly visible and required closer inspection of the jaw area for detection. A mark was recorded as absent whenever there was no evidence of externally visible color.

In the laboratory experiments with brown trout, half of the dyed fish were held at 47° F. and half at 55° F. The former were relatively slow growing, and it was rather obvious that members

of this group generally possessed marks superior to those observed for the faster growing group. All test fish examined at 28 months possessed externally visible marks, but many were badly faded. When the fish were examined at 32 months, the marks were extinct for all fish in the faster-growing group, and removal of the overlying skin layers at the injection site failed to reveal a concealed mark. For the 11 survivors in the slower-growing group, marks were absent in two fish while the remaining nine bore blue dye marks ranging from good to poor.

In a field experiment 156 brook trout were injected with National Fast Blue 8GXM in October 1964 and released in Long Pond in Franklin County. In May 1966, after 1½ years, 11 of these fish were recaptured, and all possessed visible blue dye marks only one of which was considered poor. The following fall, 12 trout were recaptured, 11 of which possessed a visible blue jaw mark. Six of these fish had good marks and five had poor marks. The mark was no longer visible on the other fish which had a 218 per cent gain in weight, the highest gain recorded for an individual fish recaptured at Long Pond. It is interesting that this trout was first recaptured at 1 year when it was 11.5 inches long and weighed 0.57 pound. At that time it possessed a good mark and had increased 103 per cent in weight.

Another field test was conducted at Laramie Pond, also in Franklin County. In June 1965, 179 brook trout averaging 4.5 inches in length and 0.04 pound in weight were injected with National Fast Blue 8GXM and released. Seven were recaptured in May 1966, about 11 months later, when they averaged 8.2 inches and 0.19 pound or a gain in weight of 375 per cent. Six marks were good and one was poor. In October 1966, 16 months after marking, nine trout were recaptured and no dye was present on any fish. They averaged 10.7 inches and 0.52 pound or a gain in weight of 1,200 per cent.

The data for these three experiments are presented in Table 1 and indicate that: (1) time itself was of minor importance as a factor affecting mark permanence; (2) all dyed fish possessed visible blue marks until the gain in weight exceeded 200 per cent; and (3) at gains of about 220 per cent some of the marks became extinct and some were still clearly visible.

It is important to note that marks were extinct on all the laboratory brown trout having an average weight gain of 322 per cent, but they were present on all brook trout from Laramie Pond when the average weight gain had reached 375 per cent. Whether this represented a true interspecific difference is unknown since a number of statistical and biological variables existed. These included

Table 1. Relationship of Mark Quality to Weight Gain at Various Time Intervals for Brown Trout and Brook Trout Jaw Injected With National Fast Blue 8GXM

Location of experiment	Gain in weight* (per cent)	Time (months)	Mark quality
	Brown trout		
Laboratory	50 (19)	12	Good
	117 (18)	12	Good
	131 (16)	24	Good
	162 (14)	28	Good to poor
	208 (13)	32	Good to absent
	248 (13)	24	Good to poor
	277 (8)	28	Poor
	322 (5)	32	Absent
	Brook trout		
Long Pond	4	6	Good
	5	12	Good
	23	6	Good
	27	12	Good
	28	24	Good
	29	18	Good
	43	6	Good
	56	12	Good
	100	18	Good
	104	12	Good
	108	18	Poor
	126	18	Good
	128	12	Good
	128	24	Good
	138	24	Poor
	141	18	Good
	148	12	Good
	156	24	Good
	176	24	Good
	196	24	Poor
	218	24	Absent
Laramie Pond	375 (7)	11	Good to poor
	1,200 (9)	16	Absent

* For laboratory records and those from Laramie Pond, figures in parentheses represent number of fish in sample. For Long Pond, each record represents a single specimen.

use of mean weight in computing the percentage gain, the small number of fish in the samples, temperature, water quality, age, size, sexual maturity and other differences.

DURABILITY OF HYDRATED CHROMIUM OXIDE

Hydrated chromium oxide is also known as "emerald green". It is insoluble in water and is unaffected by acids, alkalis, paint vehicles and solvents. In commercial applications the pigment has

proved to be stable, permanent and non-fading. It has been used as a green colorant in many preparations including cosmetics and paints, and the pure dye is generally described as $Cr_2O_6 \cdot xH_2O$. The dye is listed in the Colour Index (Society of Dyers and Colourists, 1956) as Pigment Green 18.

Chromium oxide is a similar dye and is known as "chrome green". It also has a high order of permanence and stability. Chrome green pigments occur in a range of colors from a light yellowish to a dark bluish green. Using a water paste of this material, Dunstan and Bostick (1956) successfully tatooed coho salmon fingerlings with marks that lasted for 97 days. The pure oxide is generally described as Cr_2O_3 and is listed in the Colour Index as Pigment Green 17.

Hydrated chromium oxide was tested under laboratory conditions on 40 brown trout and one brook trout. Field tests with brook trout were conducted in Long Pond. In both environments, test conditions were identical to those previously described for National Fast Blue 8GXM since the two dyes were tested simultaneously.

The results of the laboratory experiments with brown trout (Kelly, 1967) showed that the dye was present on all fish for 12 months. When examined at 16, 18, 24 and 26 months, one or more test fish failed to show evidence of an externally visible mark, and the marks were considered extinct. These fish were removed from the experiment and were not examined for concealed marks. When the remaining dyed fish were next examined at 32 months, three showed no evidence of an externally visible mark, but they proved to have well defined but concealed dye marks which were strikingly revealed when the overlying skin layers at the injection site were removed with a penknife. These fish remained alive and healthy, and the dye marks remained intact. Two of them had weight gains of 322 per cent, a degree of growth at which all of the marks had become extinct for National Fast Blue 8GXM under identical testing conditions. In the light of evidence indicating the presence of concealed marks at 32 months, it is likely that many of the fish rejected earlier also possessed concealed green dye marks. The single brook trout still displayed a good externally visible green mark at 36 months in spite of a rather prodigious gain in weight of 650 per cent.

In the field experiment conducted at Long Pond, where 132 brook trout were originally marked with hydrated chromium oxide, seven fish were recaptured 1½ years later and five were recaptured 2 years after marking. With the exception of one of those recaptured at 2 years that possessed a poor quality mark, all retained good

quality marks. The three greatest individual gains in weight for this group were 196, 195 and 182 per cent.

DISCUSSION

In the author's opinion marking trout by the shallow intracutaneous injection of dyes into the skin of the mandible has many advantages over fin clipping. Dye marking does not restrict normal swimming or feeding activity, the method is non-mutilating and the mark is easily applied. There is also considerable evidence that dye marks are more durable than fin clips. In the laboratory, dye-marked trout were originally fin clipped to provide positive identification in the event of dye loss. At 6 months, both pectoral and pelvic fins had been reclipped on many fish; otherwise a number would probably have regenerated completely within a year. Reclipping was also necessary when several other dyed fish were examined at 12 and 18 months. These same fish possessed well defined dye marks.

National Fast Blue 8GXM is a brilliant blue color, and it is very easy to inject into fish. After considerable growth (over 200 per cent) the mark becomes faded, and as more growth occurs the marks eventually become extinct. This is an excellent dye to use whenever growth rates are expected to be moderate or low during the test interval or where fast-growing populations are to be marked for short intervals. Trout marked when 8 inches long would be expected to possess visible dye marks until they reach about 12 inches. Fish that are re-examined annually, such as hatchery breeders, could easily be re-injected to renew a fading mark.

Hydrated chromium oxide produces a well defined green mark of moderate intensity. It has the disadvantage of being somewhat more difficult to inject because it is a pigment dispersion containing rather large, coarse particles, and it tends to plug needles smaller than #25. However, this dye is very durable. It has the advantage of remaining as a concealed mark which can be detected by removing overlying skin layers, and the removal process does not kill the fish. While the incidence and durability of concealed marks is presently unknown, observations from these rather limited tests show that hydrated chromium oxide was longer lasting than National Fast Blue 8GXM in situations where considerable growth occurred between the time of marking and recapture. With trained personnel available to examine recaptured fish, it would be possible to detect concealed marks several years after marking. In the light of current findings, hydrated chromium oxide seems the best prospect for a lifetime mark.

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GENERAL NOTES

MARKING SMALL TROUT BY CHEEK PAD INJECTION [1]

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One of the more difficult problems in marking fish concerns placement of durable, harmless tags or marks on small fish. Webster (1962), in an evaluation of a number of conventional methods of tagging, listed the results of 43 respondents who used more than a dozen different methods. The smallest fish reported tagged were 4 to 4½ inches long. Fin clipping is also widely used and has been employed with considerable success in marking groups of fish for migration studies, population estimates, and return records for stocked trout. It is possible to mark fish less than 4 inches long by this method. However, feeding and swimming behavior may be affected, infection may occur, and regeneration of fins often occurs. A greater incidence of regeneration would be expected the smaller the fish clipped.

Recently, dye marking has shown much promise as a method of marking small fish. Routes of administration include feeding, immersion and injection. When Sudan Black B was fed to 1- to 2-inch fry of brown trout (*Salmo trutta*) they turned blue and remained marked for about 12 days (Loeb, 1966). Immersion of 1- to 2-inch fry of brook trout (*Salvelinus fontinalis*) and brown trout in Bismarck Brown Y yielded a mark which lasted for about 3 weeks (Arnold, 1966; Kelly, unpub.). Jaw injection is excellent for marking trout 5 inches long or larger (Kelly, 1967a). But it becomes progressively more difficult as fish smaller than that are injected, and it is practically impossible to use it for trout 2½ inches or less in length. Injection of the cheek pad is a new approach and was reported by Kelly (1967a) to have provided marks lasting for 6 months on adult smallmouth bass (*Micropterus dolomieui*), pumpkinseed (*Lepomis gibbosus*), carp (*Cyprinus carpio*), yellow perch (*Perca flavescens*) and golden shiner (*Notemigonus crysoleucas*).

The mark produced by cheek-pad injection is a brilliant blotch of color irregular in size and shape and is easily seen at a distance of several feet from the fish. The cheek pad is a relatively large area and, therefore, might be suitable as an injection site for tiny fish. This paper describes an experiment to determine the usefulness of cheek-pad injection for marking brown trout fry which averaged less than 2 inches long.

Using a #27 hypodermic needle and 2½-cubic centimeter plastic syringe, 200 fingerling brown trout averaging 1.85 inches long (range 1.6 to 2.1 inches) and 0.053 ounce in weight were injected in the cheek pad with a 6.5 per cent (65 milligrams of dye per milliliter of water) solution of National Fast Blue 8GXM (formerly called Direct Fast Turquoise PT). The marking rate was 125 trout per hour. Inspection of the fish immediately after marking showed that 196 possessed readily visible blue marks, but the remaining four showed no visible mark. These fish probably received no dye at all. Every effort was made to inject very small doses, but the amount released was extremely variable. The four fish were then successfully injected. Sixty undyed fingerlings were added as controls. The fish were held in a 350-gallon fiberglass aquarium and were fed dry food at least once a day. They were removed from the aquarium, separated into test and control lots, weighed, measured and examined for dye marks at 4½, 6½ and 7 months.

Within 15 minutes after marking a mortality of 21 trout occurred, and during the first four days 26 more died, making a total early mortality of 23.5 per cent. No control fish died during this interval. Although extreme care was exercised, all losses were attributed to faulty technique including massive overdosage and injury to the gills. It was difficult to control the amount of dye injected as well as the depth of injection, and often the entire head turned blue. This was occasionally accompanied by protrusion of the eyes. Within a week following injection, the remaining trout appeared normal in all aspects of behavior including swimming, feeding response and avoidance responses. During the remainder of

[1] A contribution of Federal Aid in Fish and Wildlife Restoration Project F-9-R.

Table 1. Retention of Dye Marks for Brown Trout Fingerlings Injected in the Cheek Pad With National Fast Blue 8GXM

Time (months)	Number of fish	Number retaining mark	Average length		Average weight		Mark quality
			Inches	Gain (per cent)	Ounces	Gain (per cent)	
0	200	200	1.85	..	0.053	..	Generally good; a few poor
4½	148	148	3.93	112	0.430	711	About half good; others beginning to fade
6½	134	103	5.29	186	1.030	1,843	Generally poor and badly faded; a few good
7	131	44	5.48	196	1.270	2,296	Very faint

the experiment, the little mortality that occurred was similar to that observed among the control fish.

It has been shown that fading and eventual extinction of jaw-injection marks depend on the growth of the fish, and that growth in terms of percentage gain in weight seems to be more important than gain in length. When Kelly (1967b) injected larger trout (over 7 inches long), the fish always possessed visible marks until weight gains exceeded 200 per cent. Smaller brook trout (4½ inches long) possessed visible marks at weight gains of 375 per cent after they had been at liberty for 11 months, but on specimens captured 5 months later, and having weight gains of 1,200 per cent, the dye marks were completely extinct. It is assumed that the durability of cheek pad-injection marks also depends on the growth of the fish, but perhaps to a different degree.

The data in Table 1 show that all of the brown trout had retained their marks at 4½ months when weight gains averaged 711 per cent and the fish were about 4 inches long. When examined at 6½ months, visible marks were absent on 31 (23 per cent) of the fish, weight gains averaged 1,843 per cent, and the trout averaged about 5¼ inches long. At 7 months only a third of the fish remained dyed, most of the marks being very faded, and weight gains averaged 2,296 per cent.

Comparing these data with those obtained from the jaw injection experiments, it appears that cheek pad injections tended to persist through greater increases in growth than did jaw injections. However, this observation is complicated by differences in the species and size of the fish involved. To resolve this, it would be necessary to conduct experiments involving the two species, as well as small-sized and large-sized groups of each, and to place both cheek pad and jaw marks on the same fish.

It was fairly easy to inject small trout in the cheek pad, but, even with extreme care, some post-marking mortality occurred. This difficulty might be overcome by using a special syringe which would deliver a small, constant dose. Unfaded dye marks are readily visible at a distance of 6 feet, and it is not necessary to handle the fish to detect the mark. In fact, recapture is not necessary if good viewing conditions exist. It is unknown whether such marks represent a survival disadvantage through attraction of predators. In situations where the entire population could be recaptured at chosen intervals, it would be possible to maintain identification from the fry stage to adulthood through reinjection when marks faded. Trout fry could be cheek pad injected, recaptured at about 4 inches and then jaw injected. When the fish reached about 8 inches (about 375 per cent gain in weight) they could be examined and reinjected in the jaw. After that, growth rate would determine the frequency of injection. This technique could be used for hatchery, farm pond and aquarium populations.

Cheek pad injection with National Fast Blue 8GXM provides one of the most durable externally visible marks for the early-life stages of trout currently available. Considering that most of the fish used in this study were less than 2 inches long and that other methods of marking extremely small fish often have greater disadvantages, this seems to be a practical method of marking such fish, but its applicability is currently limited by rather high post-marking mortality. Further improvement of the technique so that shallow injections of small amounts of dye could be administered consistently would greatly extend its usefulness.

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SECOND LITTERS IN THE STRIPED SKUNK[1]

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A search of past and current literature revealed only one record of a striped skunk (*Mephitis mephitis*) producing more than a single litter in one year. A. R. Shadle (1953) reported that a captive skunk produced two litters during the same breeding season. Apparently this particular female did not lactate for the first litter, and the young died. Shadle suggested that lactation has an inhibitory effect on the next sexual cycle. Nine days after giving birth to the first litter, she was rebred and eventually produced, and successfully raised, a second litter. Other authors have indicated that the striped skunk can produce more than one litter a year or does have a second heat period if not bred, or if unsuccessfully bred, during the first heat period. But, they have cited no specific instances.

A colony of striped skunks was established at the Wildlife Research Laboratory at Delmar (N. Y.) during the summer and fall of 1965 for the purpose of studying the breeding characteristics of the species. During the 1966 breeding season two females each produced second litters after losing their first litters. Also one female which was bred but did not conceive during the first heat period produced a litter after being bred during a second heat period. Following is a description of the circumstances in each case.

1. One of the females in the colony had been box trapped locally in September 1965 when it was judged to have been 9 or 10 weeks old. On March 4, 1966, this female was placed with a large adult male (also wild trapped) in an 8- x 10-foot cage some distance from the main colony area. Each animal had a separate nest box which it accepted as its own. Twice

[1] Appreciation is expressed to George Teidman, Conservation Aide, for contributing pertinent data from the skunk breeding observations.

during daylight hours the female was observed with the male in the latter's nest box. Copulation was assumed to have taken place at night and unobserved. A litter of undetermined number (at least three) was born to the female on May 5 after a gestation period of no longer than 62 days. The female and her litter were left in the cage with the male. Within five days the entire litter disappeared, having been destroyed either by the male or by the female because of the immediate presence of the male. It was not known whether or not the female lactated.

During the second week in June the female was placed in an individual cage. She produced a litter of four on July 20, indicating that she had been rebred on May 22 plus or minus about 5 days.

2. Another female at least a year old, which had been purchased from a dealer in Iowa, was bred on March 3, 1966 and produced a litter of eight on May 4, a gestation period of 62 days. Because of improper postnatal handling and nest box facilities, the entire litter was lost, the last individual dying on May 16. This female was definitely lactating.

   On that same day, May 16, a male was placed with the female for approximately 6 hours. Although the female showed considerable belligerence toward the male, copulation was observed several times during this period. Again on June 3, the same male was placed with the female. After preliminary belligerence and foot stomping the female tolerated the male, but she was not receptive to copulation. The male was not aggressive either, indicating the female was not in receptive condition at that time. They remained together for 20 days, during which time copulation was never observed. On July 16 the female produced a litter of nine, indicating that rebreeding had occured on May 16 with a gestation period of 61 days.

3. A third female, of the same age and source as No. 2, was observed to breed on March 4, 1966 but failed to produce a litter. She was placed with a male again from May 22 to May 26. They were observed to copulate several times on May 22. On July 20 she produced a litter of five, indicating that conception probably occurred on the first day of the rebreeding period, May 22. If this was the case, the gestation period would have been 59 days, the shortest recorded for this colony.

From the experience and observations recorded during the 1966 breeding season, multiple litters are quite possible for the striped skunk. Lactation does not seem to inhibit copulation or pregnancy. Additional studies are being carried on to determine whether or not a skunk may produce and raise two healthy litters in a single breeding season.

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